USER GUIDE

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Applied Biosystems SOLiD® 4 System

INSTRUMENT OPERATION GUIDE

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About This Guide

Note: For general safety information, see the "Safety" appendix in this document. For important safety information related to Corvaris® S2 system, please refer to the user documentation of the product. When a hazard symbol and hazard type appear by a chemical name or instrument hazard, see the "Safety" appendix for the complete alert on the chemical or instrument.

CAUTION! ABBREVIATED SAFETY ALERTS. Hazard symbols and hazard types specified in procedures may be abbreviated in this document. For the complete safety information, see the "Safety" appendix in this document.

User attention words

Five user attention words may appear in this document. Each word implies a particular level of observation or action as described below:

Note: Provides information that may be of interest or help but is not critical to the use of the product.

IMPORTANT! Provides information that is necessary for proper instrument operation or accurate chemistry kit use.

CAUTION! Indicates a potentially hazardous situation that, if not avoided, may result in minor or moderate injury. It may also be used to alert against unsafe practices.

WARNING! Indicates a potentially hazardous situation that, if not avoided, could result in death or serious injury.

DANGER! Indicates an imminently hazardous situation that, if not avoided, will result in death or serious injury.

Except for IMPORTANTs, the user attention words in user documentation appear with an open triangle figure that contains a hazard symbol. These hazard symbols are identical to the hazard symbols that are affixed to the instrument. See the "Safety" appendix for descriptions of the symbols.

1 Introduction

SOLiD® 4 System run types

On the Applied Biosystems SOLiD® 4 System, you can perform a workflow analysis (WFA) run and a sequencing run (see Table 1).

Table 1 Run types on the SOLiD® 4 System

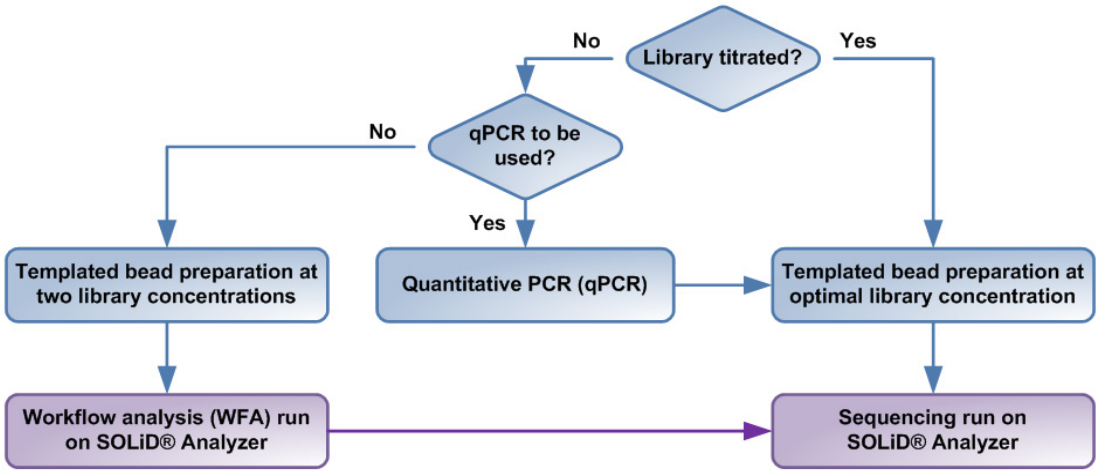
	WFA	Sequencing
Purpose	• Assess various preparations of templated beads to determine the potential quality of sequence data • Evaluate the fraction of P2-positive beads • Use as a tool to determine the deposition density for sequencing slides	Generate sequencing data for fragment or mate-paired libraries
Run summary	• P1 and P2 bead counting • Single ligation cycle • Report generation	Multiple ligation cycles for each of 5 primers, resulting in up to 50 bases of sequence information per tag†
Estimated run time‡	~4–5 hours	• ~4–14 days
Deposition chamber	4-well	• 1-well • 4-well • 8-well
Number of beads	15 million beads per well	• 708 million beads per well (1-well) • 128 million beads per well (4-well) • 56 million beads per well (8-well)

† One tag for fragment sequencing and two tags for mate-pair sequencing and paired-end sequencing. The reverse read tag in paired-end sequencing is currently limited to 25 bases. Only 5 or 10 bases of the barcode tag need to be sequenced.

‡ Total run time for dual slide run. Run time varies with read length and type of sequencing run. Times may deviate depending on imaging time.

Use Figure 1 to choose the run type that most closely meets your sequencing needs:

Figure 1 Relationship between the workflow analysis (WFA) and sequencing runs on the SOLiD® System.



Workflow analysis (WFA) run

You can optimize sequencing results by performing workflow analysis (WFA) runs. A WFA run analyzes a quadrant of a slide that undergoes a single ligation cycle. The quadrant contains beads deposited at a lower density than the density of beads deposited for a sequencing run.

A WFA run determines the:

- *Optimal library concentration*: the library concentration for optimal preparation of templated beads using the library. You use this library concentration for any preparation of templated beads for that library as long as the scale of templated bead preparation is the same.
- *Bead enrichment efficiency*: the proportion of beads that have been successfully amplified using emulsion PCR (ePCR) as a fraction of the total number of beads prepared. You use this value to accurately deposit successfully amplified beads for a sequencing run.

WFA runs require the same materials as those materials needed for sequencing runs. If you perform multiple WFA runs routinely, you should order additional SOLiD® Instrument Buffer Kits.

To perform a WFA run, prepare slide and install reagents according to the procedure in Chapter 2, "Prepare and Install Slides and Reagents" on page 17, then set up and monitor the run according to the procedure in Chapter 3, "Set Up, Control, and Monitor the Run" on page 43.

Sequencing run

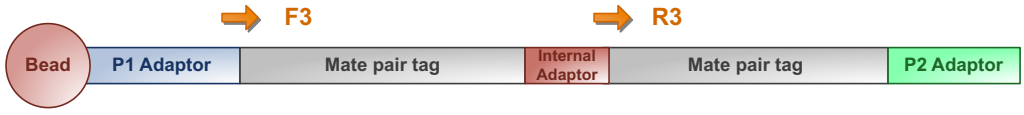
There are five types of sequencing runs that can be performed on the SOLiD® 4 System: fragment sequencing, paired-end sequencing, mate-pair sequencing, multiplex fragment sequencing, and multiplex paired-end sequencing (see Figure 2).

Figure 2 Types of sequencing runs.

Fragment sequencing

Paired-end sequencing

Mate-pair sequencing



Multiplex fragment sequencing

Multiplex paired-end sequencing

The type of sequencing run depends on the type of library to be sequenced (see Table 2).

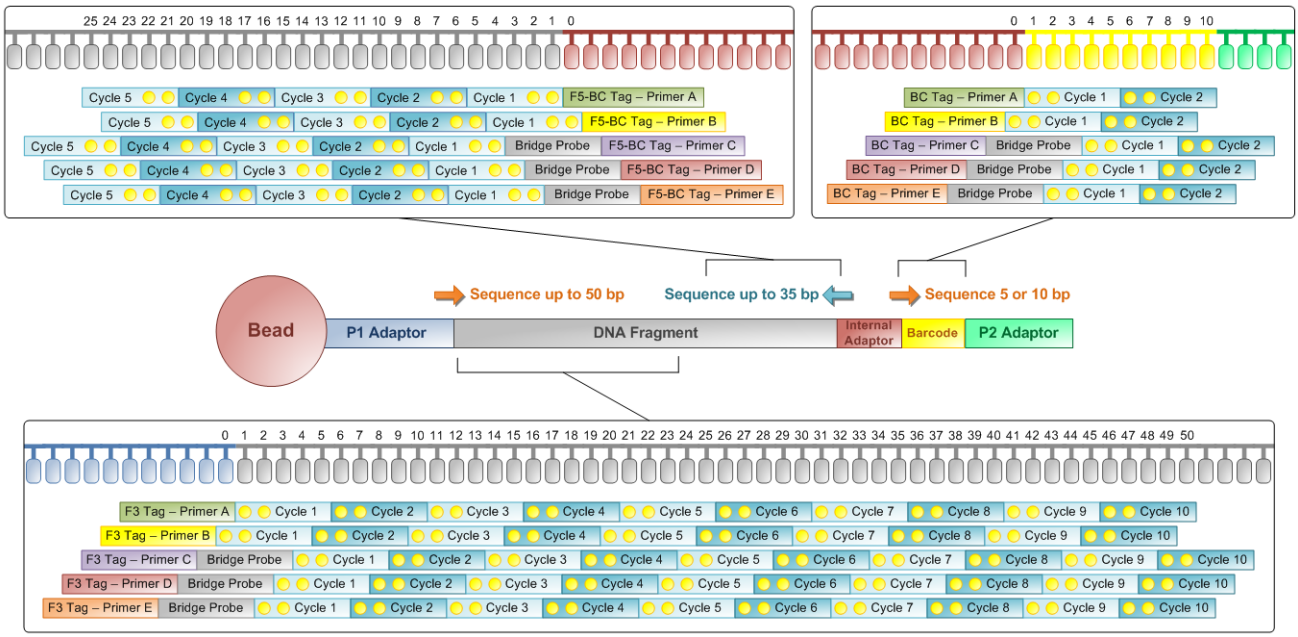
Table 2 Types of sequencing runs possible for each library type

Library type	Possible sequencing run type(s)
Fragment library	• Fragment sequencing • Paired-end sequencing
Mate-paired library	• Mate-pair sequencing
Barcoded fragment library	• Multiplex fragment sequencing • Multiplex paired-end sequencing

During a SOLiD® sequencing run, two probe sets are used to maximize the fraction of mappable beads, read length, and sequencing throughput. (Mappable beads are beads amplified with template that map to the reference genome.)

Compared to terminator-based sequencing chemistry, with SOLiD® System sequencing, base information is not collected; instead, five rounds of primers (Primers A, B, C, D, and E) are used to sequence template by ligation of di-base labeled probes for each tag (for examples, see Figure 3).

Figure 3 Di-base sequencing in a mate-pair sequencing run (top) and in a multiplex paired-end sequencing run (bottom).


25 24 23 22 21 20 19 18 17 16 15 14 13 12 11 10 9 8 7 6 5 4 3 2 1 0
Cycle 5 Cycle 4 Cycle 3 Cycle 2 Cycle 1 F5-BC Tag – Primer A
Cycle 5 Cycle 4 Cycle 3 Cycle 2 Cycle 1 F5-BC Tag – Primer B
Cycle 5 Cycle 4 Cycle 3 Cycle 2 Cycle 1 Bridge Probe F5-BC Tag – Primer C
Cycle 5 Cycle 4 Cycle 3 Cycle 2 Cycle 1 Bridge Probe F5-BC Tag – Primer D
Cycle 5 Cycle 4 Cycle 3 Cycle 2 Cycle 1 Bridge Probe F5-BC Tag – Primer E
0 1 2 3 4 5 6 7 8 9 10
BC Tag – Primer A Cycle 1 Cycle 2
BC Tag – Primer B Cycle 1 Cycle 2
BC Tag – Primer C Bridge Probe Cycle 1 Cycle 2
BC Tag – Primer D Bridge Probe Cycle 1 Cycle 2
BC Tag – Primer E Bridge Probe Cycle 1 Cycle 2
Sequence up to 50 bp
Sequence up to 35 bp
Sequence 5 or 10 bp
Bead
P1 Adaptor
DNA Fragment
Internal Adaptor
Barcode
P2 Adaptor
0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29 30 31 32 33 34 35 36 37 38 39 40 41 42 43 44 45 46 47 48 49 50
F3 Tag – Primer A Cycle 1 Cycle 2 Cycle 3 Cycle 4 Cycle 5 Cycle 6 Cycle 7 Cycle 8 Cycle 9 Cycle 10
F3 Tag – Primer B Cycle 1 Cycle 2 Cycle 3 Cycle 4 Cycle 5 Cycle 6 Cycle 7 Cycle 8 Cycle 9 Cycle 10
F3 Tag – Primer C Bridge Probe Cycle 1 Cycle 2 Cycle 3 Cycle 4 Cycle 5 Cycle 6 Cycle 7 Cycle 8 Cycle 9 Cycle 10
F3 Tag – Primer D Bridge Probe Cycle 1 Cycle 2 Cycle 3 Cycle 4 Cycle 5 Cycle 6 Cycle 7 Cycle 8 Cycle 9 Cycle 10
F3 Tag – Primer E Bridge Probe Cycle 1 Cycle 2 Cycle 3 Cycle 4 Cycle 5 Cycle 6 Cycle 7 Cycle 8 Cycle 9 Cycle 10

The workflow for each type of sequencing run is shown in Figure 4.

Figure 4 Sequencing run workflows.

Fragment sequencing
Load reagents and slide onto SOLiD® Analyzer
Run F3 Tag – Primers A to E
(up to 10 cycles per primer)
Instrument completes the run
Paired-end sequencing
Load reagents and slide onto SOLiD® Analyzer
Run F5-P2 Tag – Primers A to E
(up to 7 cycles per primer)
Instrument pauses
Load reagents onto SOLiD® Analyzer
Run F3 Tag – Primers A to E
(up to 10 cycles per primer)
Instrument completes the run
Mate-pair sequencing
Load reagents and slide onto SOLiD® Analyzer
Run R3 Tag – Primers A to E
(up to 10 cycles per primer)
Instrument pauses
Load reagents onto SOLiD® Analyzer
Run F3 Tag – Primers A to E
(up to 10 cycles per primer)
Instrument completes the run
Multiplex fragment sequencing
Load reagents and slide onto SOLiD® Analyzer
Run BC Tag – Primers A to E
(1 or 2 cycles per primer)
Instrument pauses
Load reagents onto SOLiD® Analyzer
Run F3 Tag – Primers A to E
(up to 10 cycles per primer)
Instrument completes the run
Multiplex paired-end sequencing
Load reagents and slide onto SOLiD® Analyzer
Run BC Tag – Primers A to E
(1 or 2 cycles per primer)
Instrument pauses
Load reagents onto SOLiD® Analyzer
Run F5-BC Tag – Primers A to E
(up to 7 cycles per primer)
Instrument pauses
Load reagents onto SOLiD® Analyzer
Run F3 Tag – Primers A to E
(up to 10 cycles per primer)
Instrument completes the run

Software operation and data analysis

The SOLiD® 4 System comprises multiple complementary analysis software components that complete primary analysis (image acquisition, signal processing, color calling, and quality control) and secondary analysis (alignment to a reference genome, SNP identification, and base calling) of fragment, mate-pair, and paired-end sequencing experiments.

For information describing the relationship between ICS (SOLiD® Instrument Control Software), SETS (SOLiD® Experimental Tracking System), and BioScope Software, refer to the *Applied Biosystems SOLiD® 4 Software Operation and Data Analysis Quick Reference Guide* (Part no. 4448432).

For additional secondary and tertiary analysis tools, visit the SOLiD® Software Development Community website (**http://solidsoftwaretools.com**). You can integrate standalone tools from the SOLiD® Software Development Community with BioScope to perform more automated analysis. For details, refer to the *BioScope™ Software for Scientists Guide* (Part no. 4448431).

2 Prepare and Install Slides and Reagents

This chapter covers:

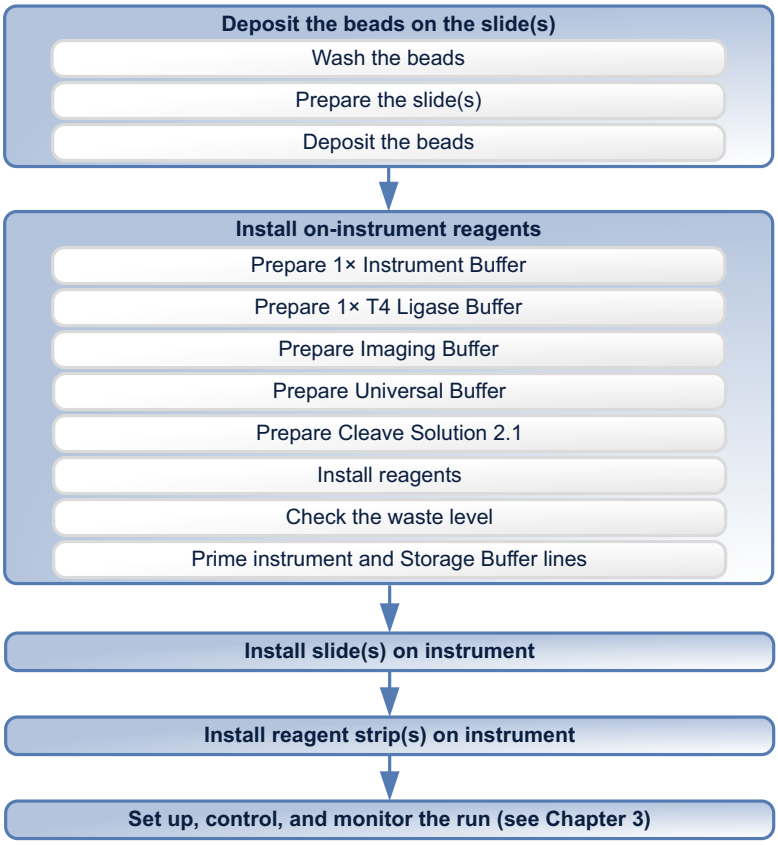
- Materials and equipment required 17
- Workflow 18
- Tips 19
- Deposit the beads on the slides 20
- Install on-instrument reagents 25
- Install slide(s) on the instrument 31
- Install reagent strip(s) on the instrument 36

Materials and equipment required

See Appendix A, "Applied Biosystems SOLiD® 4 System Instrument Operation Guide Required Materials" on page 83 for a list of equipment, kits, and consumables necessary to set up a workflow analysis (WFA) run.

See Appendix A, "Applied Biosystems SOLiD® 4 System Instrument Operation Guide Required Materials" on page 83 for a list of equipment, kits, and consumables necessary to set up a sequencing run.

Workflow


Deposit the beads on the slide(s)
Wash the beads
Prepare the slide(s)
Deposit the beads
Install on-instrument reagents
Prepare 1× Instrument Buffer
Prepare 1× T4 Ligase Buffer
Prepare Imaging Buffer
Prepare Universal Buffer
Prepare Cleave Solution 2.1
Install reagents
Check the waste level
Prime instrument and Storage Buffer lines
Install slide(s) on instrument
Install reagent strip(s) on instrument
Set up, control, and monitor the run (see Chapter 3)

Workflow overview

Deposit the beads on the slide(s)

For a WFA run, the beads are quantitated using the *Applied Biosystems SOLiD® Bead Concentration Chart* (Part no. 4415131), and 15 million beads are deposited in one well of a 4-Well SOLiD® Deposition Chamber.

For a sequencing run, the choice of SOLiD® Deposition Chamber depends on factors such as the requirements of the experiment, number of libraries being assessed, the size of the genome, and the sequencing coverage required. Three SOLiD® Deposition Chamber designs are available for use (see Table 3).

Table 3 Three deposition chamber designs

Deposition chamber	Number of image panels
1-Well	2357
4-Well	426 per well
8-Well	186 per well

Install on-instrument reagents

Prepare 1X Instrument Buffer using glycerol and 10X Instrument Buffer provided in the SOLiD® Instrument Buffer Kit. You may formulate 1X Instrument Buffer in 8-L batches as needed or prepare it in larger volumes and store at 4°C until ready for use.

Prepare T4 Ligase Buffer, Imaging Buffer, Universal Buffer, and Cleave Solution 2.1 by combining the two parts provided in the SOLiD® ToP Instrument Buffer Kit.

Cool the chiller block prior to installation of buffers.

Because the tubing from the Instrument and Storage Buffer bottles to the flowcell is long, prime the lines before installing the slides in the flowcell.

Install slide(s) on the instrument

Remove the slide from the Deposition Chamber and prepare it for installation on the instrument. Each flowcell can be loaded with the slide *independently* of each other.

Install reagent strip(s) on the instrument

Install the workflow analysis or sequencing reagent strips on the reagent strip chiller block. Cool the chiller block prior to installation of reagent strips.

Tips

General

- Prior to deposition, store the slides at –20°C to ensure optimal bead deposition and to minimize loss of P2-enriched beads.
- Remove the slides from storage at –20°C 5 minutes prior to use.

  If only one slide from the two-pack is used, place the remaining slide back into the box and place the box back into the resealable pouch. The pouch should be resealed and stored at –20°C. The stored slide should be used within 1 month.
- Use Eppendorf LoBind® Tubes to perform all steps requiring 0.5-mL, 1.5-mL, and 2.0-mL tubes. Eppendorf LoBind® Tubes from other vendors may have a chemical coating that can have adverse effects on bead deposition.
- Adjust microcentrifuge speeds and times according to the g-forces specified in the protocols (160 × g, 167 × g).

Covaris® S2 System

- The procedures are optimized for the Covaris® S2 System. The Covaris® S2 System must be specially adapted to prepare beads for the Life Technologies SOLiD® 4 System. Do not use the Covaris® S1 sonicator or an unadapted Covaris® S2 System for bead preparation. For more information, contact a Life Technologies SOLiD® System Applications Specialist.
- Ensure that the Covaris® S2 System is degassed, that bubbles are not present in the system, and that the instrument and tube are properly aligned for appropriate sonication of beads.
- To ensure optimal sonication by the Covaris® S2 System, use the appropriate adaptor with the Covaris® S2 System. For sample volumes ≤ 200 µL, use a 0.5-mL Eppendorf LoBind® Tube and 0.65-mL tube adaptor. For sample volumes between 200 µL and 600 µL, use a 1.5-mL Eppendorf LoBind® Tube and 1.5-mL

tube adaptor. For sample volumes between 600 µL and 1.2 mL, use a 2.0-mL Eppendorf LoBind® Tube and the same adaptor as used for the 1.5-mL tubes. Place the tube collar at the indicator line of the adaptor. Ensure that the white line on the tube holder is visible.

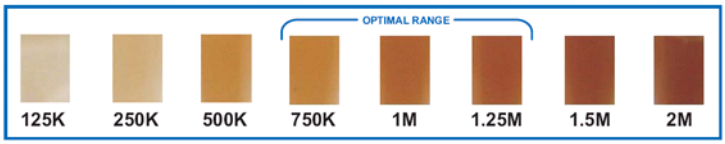
Deposit the beads on the slides

Wash the beads

Note: The bead wash procedure is for one WFA or sequencing run sample.

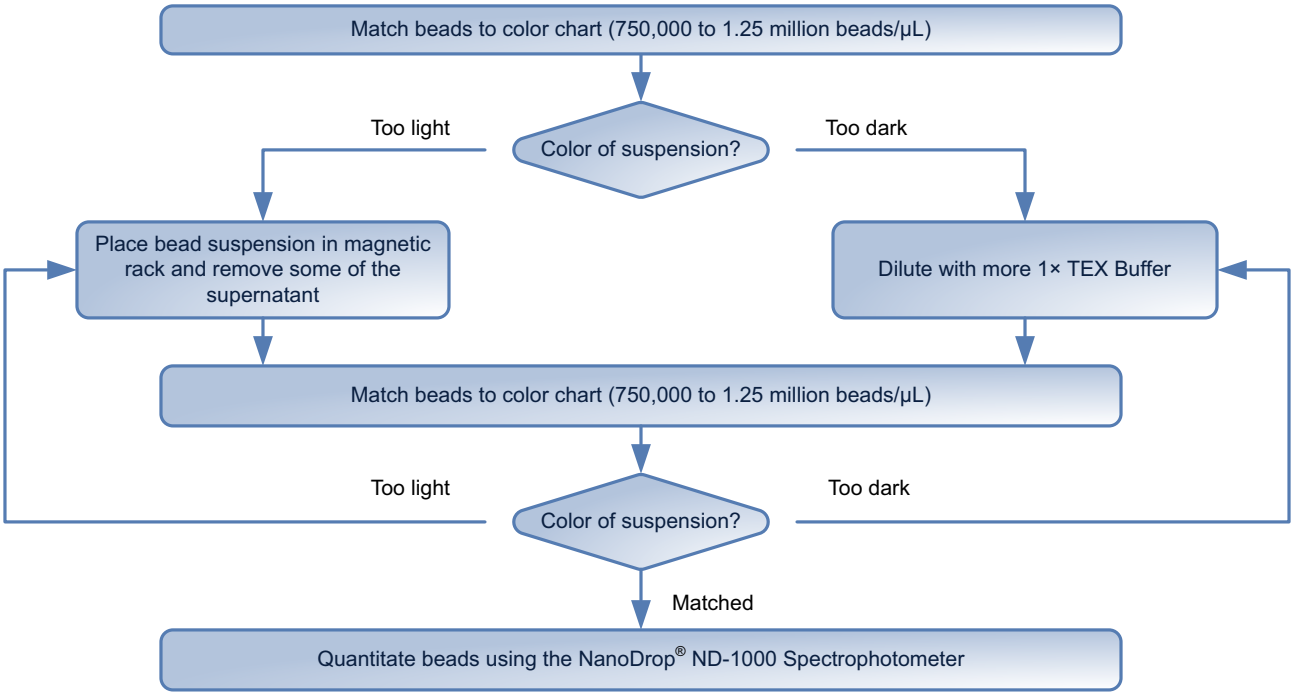
1. Sonicate P2-enriched beads using the Covalent Declump 1 program on the Covaris® S2 System (for program conditions, see Appendix G, "Covalent Declump 1" on page 142). Pulse-spin, but do not pellet the beads.
2. If a WFA run has already been performed, use the results from the WFA report to estimate the bead concentration and proceed to step 5; otherwise, use the *Applied Biosystems SOLiD® Bead Concentration Chart* (Part no. 4415131) to estimate the bead concentration (see Figure 5).

Figure 5 The SOLiD® Bead Concentration Chart. For best results, use the *Applied Biosystems SOLiD® Bead Concentration Chart* (Part no. 4415131), supplied separately.



3. Adjust the volume of beads so that the color of the bead solution matches a color in the optimal range (750,000–1.25 million beads/µL; see Figure 5 and Figure 6).

Figure 6 SOLiD® Bead Concentration Chart workflow.


Match beads to color chart (750,000 to 1.25 million beads/µL)
Too light
Color of suspension?
Too dark
Place bead suspension in magnetic rack and remove some of the supernatant
Dilute with more 1× TEX Buffer
Match beads to color chart (750,000 to 1.25 million beads/µL)
Too light
Color of suspension?
Too dark
Matched
Quantitate beads using the NanoDrop® ND-1000 Spectrophotometer

4. When the bead concentration is within the accurate range, quantitate the beads using the NanoDrop® ND-1000 Spectrophotometer. Refer to "Quantitate the beads using the NanoDrop® ND-1000 Spectrophotometer," in the *Applied Biosystems SOLiD® 4 System Templated Bead Preparation Guide* (Part no. 4448378).

5. Transfer the appropriate number of beads to a 1.5-mL Eppendorf LoBind® Tube and store the remaining beads at 4°C.

Table 4 Number of beads to use according to the type of run and deposition chamber

Type of run	SOLiD® Deposition Chamber	Target number of P2-positive beads per well	Maximum threshold number of beads per well
WFA	4-Well	15 million	30 million
Sequencing	1-Well	708 million	778 million
Sequencing	4-Well	128 million	141 million
Sequencing	8-Well	56 million	61 million

Target number of P2-positive beads per well

The targeted bead deposition density is 300,000 P2-positive beads per panel, and the maximum threshold bead deposition density for all beads (P2-positive or not) is 330,000 beads per panel. Exceeding the maximum threshold number of beads per well may result in decreased sequence quality.

Bead deposition densities may deviate from the targeted bead deposition density of 300,000 P2-positive beads per panel due to variability in the bead quantitation process.

The calculated bead concentration based on the WFA report is the most accurate because it specifically measures the concentration of P2-positive beads (see Chapter 3, "Determine the bead deposition density for a sequencing run" on page 55). If WFA run results are not available, it is possible to estimate the bead concentration using the SOLiD® Bead Concentration Chart and NanoDrop® spectrometer measurement.

It is recommended you target an additional overage volume to account for measurement variability, especially for off-instrument bead quantitation.

Typical overages can range as high as 20–50% and can vary with the library, operator, sample type, and other factors.

6. Place the tube of aliquoted beads in a magnetic rack for at least 1 minute. After the solution clears, remove and discard the supernatant.

7. Remove the SOLiD® XD slide(s) from –20°C storage to equilibrate at room temperature.

8. Resuspend the beads in 400 µL of SOLiD® XD Slide Deposition Buffer v2. Vortex thoroughly, then pulse-spin.

IMPORTANT! Do not pellet the beads.

9. Place the tube in a magnetic rack for at least 1 minute. After the solution clears, remove and discard the supernatant.

   Note: It may take longer than 1 minute for the solution to clear. If necessary, slowly pipet the solution up and down in the tube until the beads pellet against the magnet.

10. Repeat steps step 8 and step 9 *twice*.

11. Resuspend the beads in the volume of SOLiD® XD Slide Deposition Buffer v2 listed in Table 5 (calibration of the SOLiD® Deposition Chamber with the SOLiD® 4 Slide Carrier or SOLiD® Opti Slide Carrier).

IMPORTANT! For upgraded systems with new slide carriers, the SOLiD® Deposition Chamber should be recalibrated using the SOLiD® 4 Slide Carrier to determine the exact deposition volume.

Table 5 Approximate Deposition Buffer volumes

Deposition chamber	Volume per well (µL)
1-Well	550
4-Well	400
8-Well	300

Note: Deposition Buffer volume is dependent on the slide carrier as well as the Deposition Chamber, and volumes vary from slide carrier to slide carrier. In addition, residual SOLiD® XD Slide Deposition Buffer v2 that remains on the surface of the slide after washing may alter the volume slightly.

Prepare the slide(s)

1. Thoroughly clean, rinse, and dry the SOLiD® Deposition Chamber before deposition:

   a. Clean the SOLiD® Deposition Chamber by rinsing it with MilliQ water.

      Note: Do not wash the SOLiD® Deposition Chamber with ethanol because ethanol damages the adhesive on the O-ring.

   b. Blot the SOLiD® Deposition Chamber dry on a lab wipe. Be particularly careful to dry around the O-ring and to remove fluid from the fill ports.

2. Insert a new slide into the SOLiD® 4 or Opti Slide Carrier (see Figure 7).

IMPORTANT! Do not touch the slide surface.

IMPORTANT! The SOLiD® 4 and Opti Slide Carriers are painted blue across the side (see Figure 7). If you are using the SOLiD® ToP reagent strips for sequencing, you must use either the SOLiD® 4 Slide Carrier or the SOLiD® Opti Slide Carrier. Using a different slide carrier may lead to run failure.

---

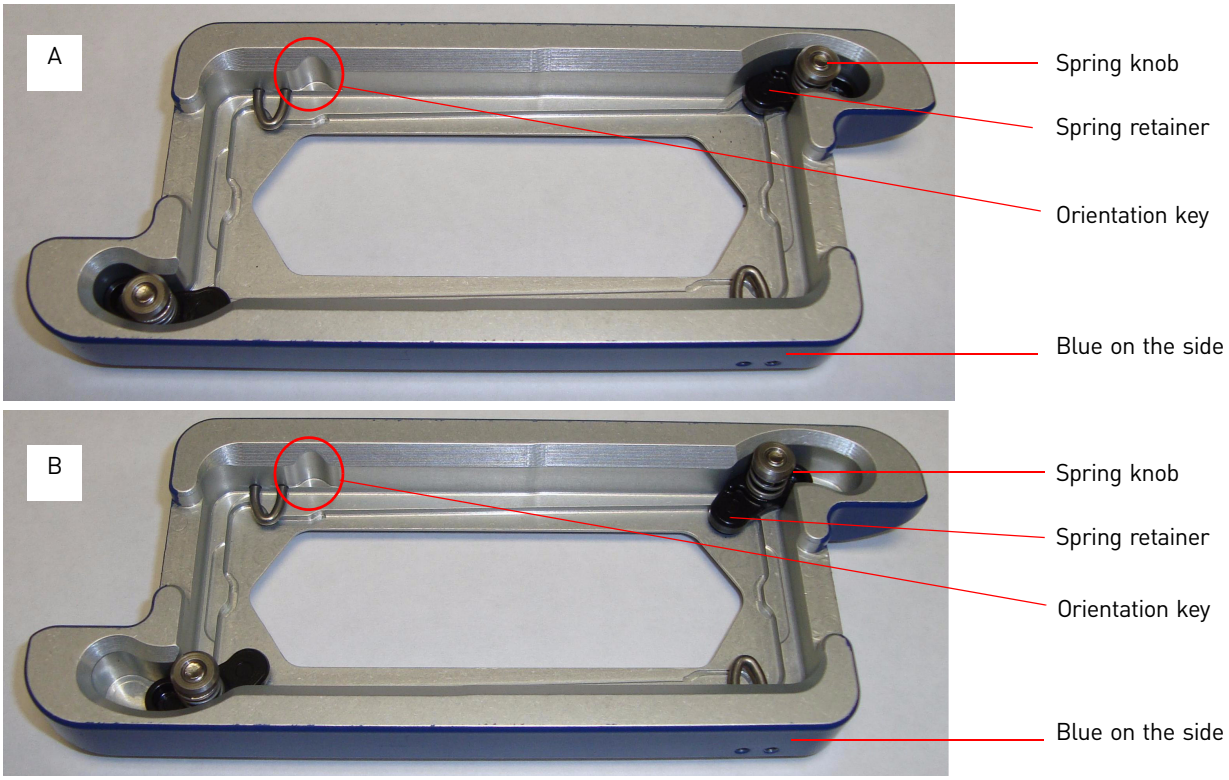
   a. Move the retainers out so that the slide fits into the SOLiD® 4 or Opti Slide Carrier. To do this, push down on the two spring knobs in the SOLiD® 4 or Opti Slide Carrier and slide the knobs towards the outside edges of the carrier (see Figure 7 A).

b. Place the slide against the alignment nubs in the SOLiD® 4 or Opti Slide Carrier.

c. Ensure that the slide is precisely positioned in the SOLiD® 4 or Opti Slide Carrier and then slide the retainers inward until they hold the slide in position (see Figure 7 B).

Note: To move the retainers over the slide, do not push down the knobs.

Figure 7 (A) The SOLiD® 4 Slide Carrier with spring knobs and retainers pushed out and (B) with spring knobs and retainers pushed in (the slide is held under the retainers).

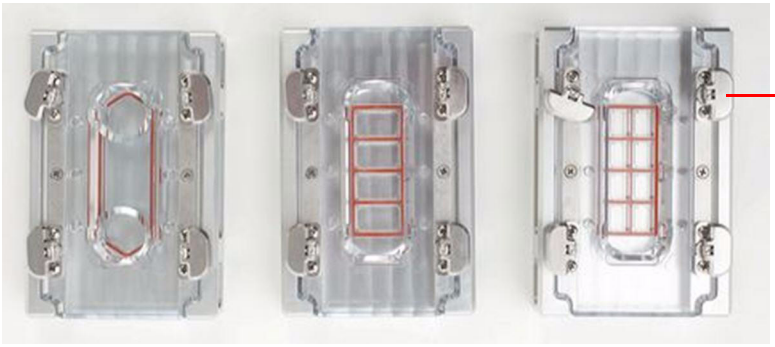


3. Immediately place the SOLiD® 4 or Opti Slide Carrier assembly into SOLiD® Deposition Chamber base, then place the appropriate SOLiD® Deposition Chamber lid on top (see Figure 8).

IMPORTANT! After washing, do not let the slide sit for more than 10 minutes before depositing beads.

Note: The SOLiD® Deposition Chamber top must engage with the orientation key to fit properly. The orientation key is in the lower-left position when the slide carrier assembly is placed on the instrument. This orientation must be maintained in order to preserve the correct sample order when the 4-Well or 8-Well SOLiD® Deposition Chamber is used.

Figure 8 SOLiD® Deposition Chambers: 1-Well, 4-Well, and 8-Well.



4. Tighten the four screw tabs on the SOLiD® Deposition Chamber in a crisscross pattern until the lid is securely attached.
5. Twist the tabs flat.

Deposit the beads

1. Sonicate the beads using the Covalent Declump 3 program on the Covaris® S2 System (for program conditions, see Appendix G, "Covalent Declump 3" on page 142). Afterward, pulse-spin, but do not pellet the beads.
2. Repeat step 1.
3. Use a pipettor with an appropriate tip to pipet the bead solution up and down a few times, then withdraw the sample of templated beads from the microcentrifuge tube.

IMPORTANT! Samples must be deposited onto the slide immediately after sonication to minimize clumping and maximize monolayering.

4. Perform one of the following sequences:

If the SOLiD® Deposition Chamber has...	Then perform steps...
1 well	5, 6, and 9 to 11
4 or 8 wells	7 to 11

5. Elevate and tilt the SOLiD® Deposition Chamber with the entry porthole of the well at the lowest point.
6. Carefully pipet a sample of templated beads into the well through the porthole. As the area of the well fills, lower the top of the SOLiD® Deposition Chamber so that it becomes level. Proceed to step 9.
7. With the SOLiD® Deposition Chamber *flat*, carefully pipet a sample of templated beads into one of the 4 or 8 wells through the porthole.
8. Repeat step 7 to fill each of the remaining wells with each sample of templated beads. Note each sample's well position relative to the slide orientation.
9. Place 3-mm adhesive disks over all the portholes in the SOLiD® Deposition Chamber.
10. Centrifuge the slide in the SOLiD® Deposition Chamber at 167 × g for 10 minutes in a plate centrifuge.

11. Incubate the SOLiD® Deposition Chamber at 37°C for 1 hour.
12. After incubation, centrifuge the slide in the SOLiD® Deposition Chamber at 167 × g for 10 minutes in a plate centrifuge.
13. For high density depositions, wash the slides with SOLiD® XD Slide Deposition Buffer v2 or SOLiD® XD Slide Storage Buffer to gently remove unbound beads prior to instrument loading.

Install on-instrument reagents

Note: For information about recommended fill volumes for on-instrument reagents, see Appendix C, "Recommended fill volumes for on-instrument reagents" on page 121.

Prepare 1X Instrument Buffer

Note: Prepare buffers just prior to use on the SOLiD® 4 Analyzer.

1. Add 800 mL of 10X Instrument Buffer to an empty 8-L reagent bottle.

IMPORTANT! Regular cleaning of the 8-L Instrument Buffer bottle is required for every run (see Appendix B, "Clean the Instrument Buffer bottle" on page 97). Failure to clean the Instrument Buffer bottle regularly may allow microbial contaminants to proliferate in the system. Never top off the Instrument Buffer bottle.

2. Add 1600 mL of glycerol to the reagent bottle. Use a graduated cylinder to measure the glycerol.
3. Add 5600 mL of double-distilled water, rinsing residual glycerol from the graduated cylinder.
4. Using a clean magnetic stir bar, mix the solution for 10 minutes to ensure homogeneity.
5. Remove the stir bar and install the prepared buffer on the SOLiD® 4 Analyzer or store at 4°C until ready for use.

Prepare 1X T4 Ligase Buffer

1. Transfer the contents of the 1X T4 Ligase Buffer Part 1 tube to the 1X T4 Ligase Buffer Part 2 bottle.
2. Gently mix the contents by slowly inverting the bottle 3 to 5 times to ensure thorough mixing and to minimize bubbles.

Prepare Imaging Buffer

1. Transfer the contents of the Imaging Buffer Part 1 bottle to the Imaging Buffer Part 2 bottle.
2. Gently mix the contents by slowly inverting the bottle 3 to 5 times to ensure thorough mixing and to minimize bubbles.

Prepare Universal Buffer

1. Transfer the contents of the Universal Buffer Part 1 bottle to the Universal Buffer Part 2 bottle.

2. Gently mix the contents by slowly inverting the bottle 3 to 5 times to ensure thorough mixing and to minimize bubbles.
3. After mixing, apply the Universal Buffer label supplied with the bottle.

Prepare Cleave Solution 2.1

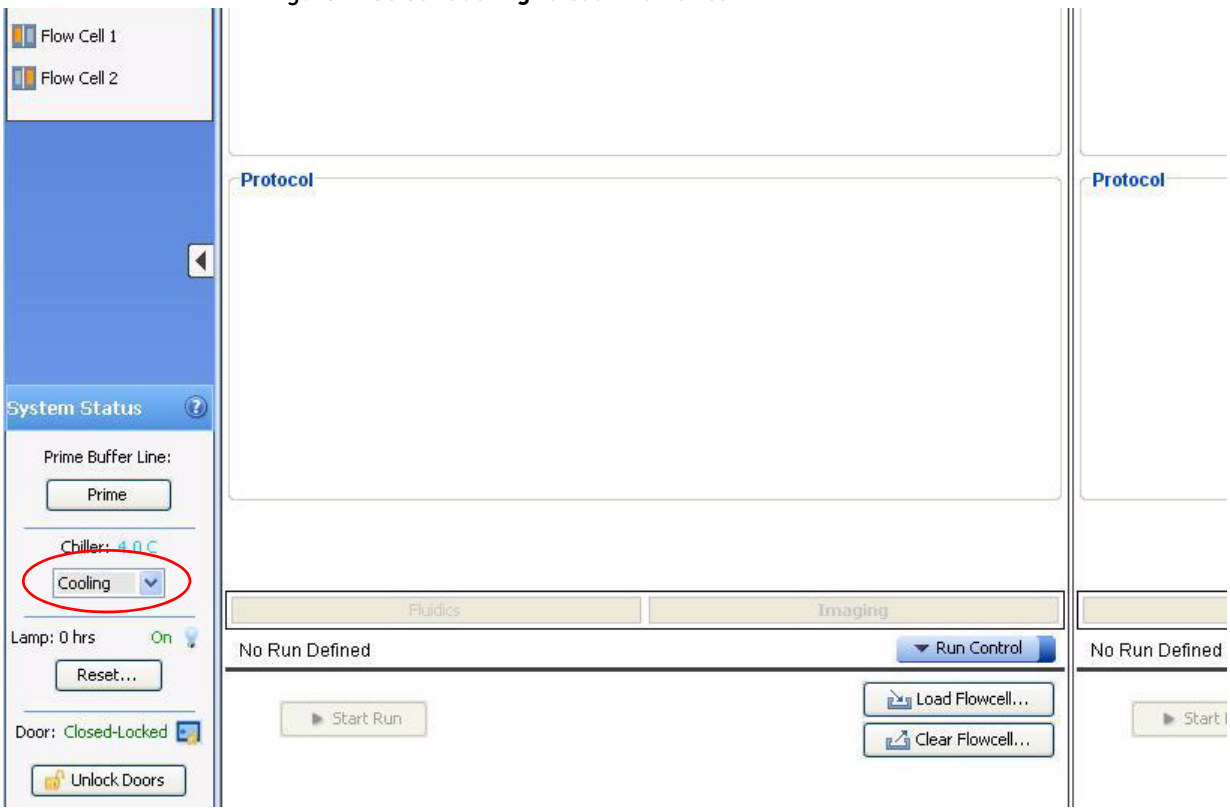
1. Transfer the contents of the Cleave Solution 2.1 Part 1 bottle to the contents of the Cleave Solution 2.1 Part 2 bottle.
2. Gently mix the contents by slowly inverting the bottle 3–5 times to ensure thorough mixing and to minimize bubbles.

Install reagents

1. If needed, flush the tubing of the SOLiD® 4 Analyzer fluidics system (see Appendix B, "Flush the fluidic lines" on page 99).

   Note: If the SOLiD® 4 Analyzer is in continuous use, the fluidics system should be flushed every three months. If the SOLiD® 4 Analyzer will sit idle for more than two weeks, the fluidics system should be flushed and the instrument powered down with the fluidics lines empty.
2. Double-click the SOLiD® **Instrument Control Software** icon to launch the SOLiD® Instrument Control Software, if it is not already open.
3. Under the System Status menu, select **Cooling** from the Chiller drop-down menu (see Figure 9).

Figure 9 Select **Cooling** to cool the flowcell.



4. After the chiller temperature is <10°C, install the prepared 1X Instrument Buffer and Storage Buffer into the appropriate positions in the cabinet (see Figure 10).

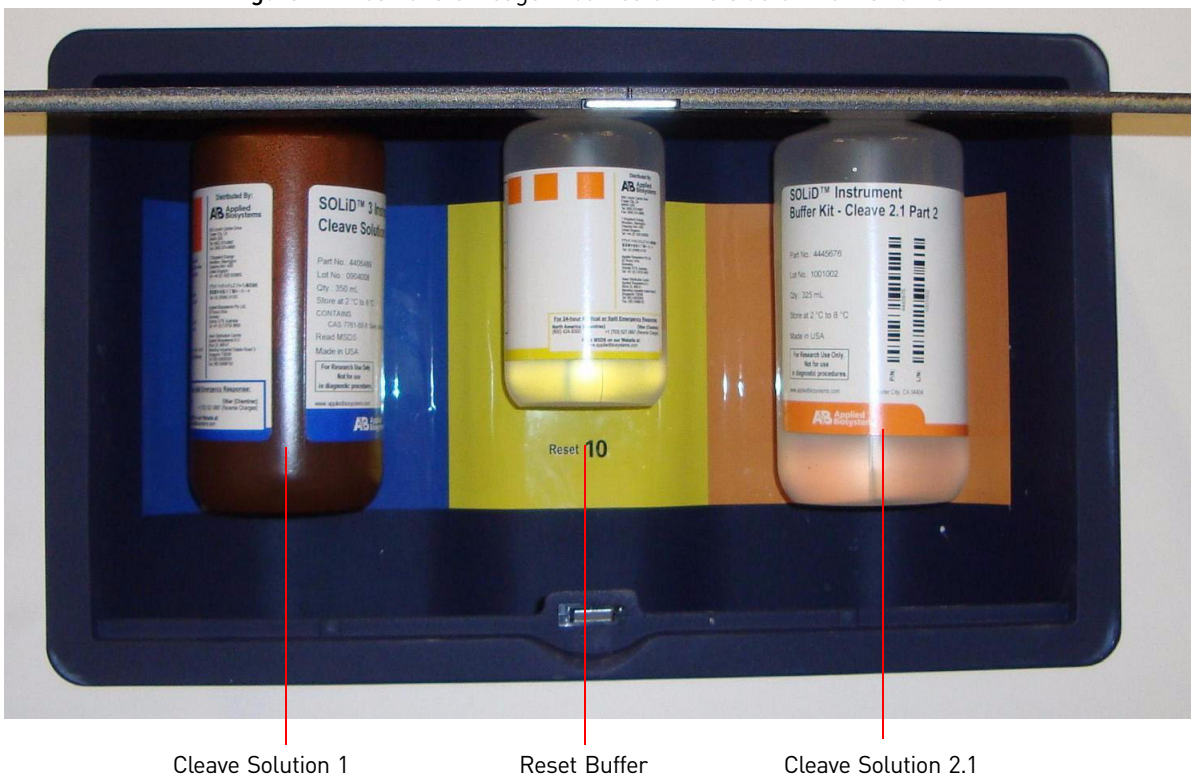
CAUTION! POTENTIAL OVERHEAD HAZARD. Use caution when working inside the cabinet.

Figure 10 Positions of buffers and waste in the cabinet.

5. Install Cleave Solution 1, prepared Cleave Solution 2.1, and Reset Buffer in the appropriate positions on the side of the instrument (see Figure 11). Make sure that the tubes on the caps of the reagent bottles are fully screwed on.

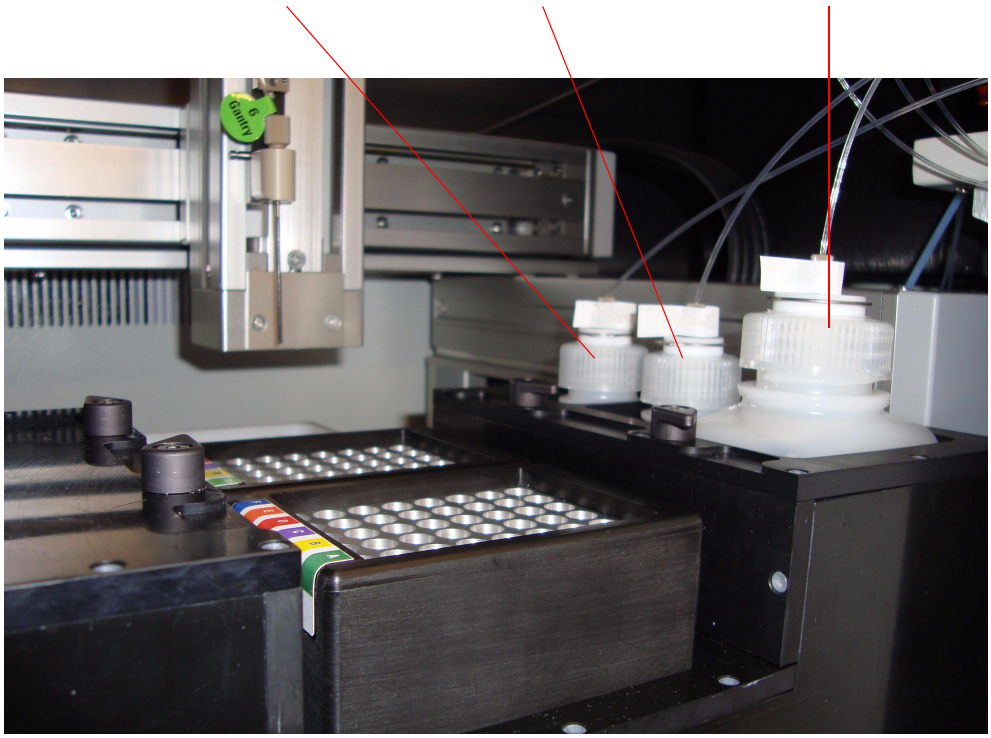
> **CAUTION!** POTENTIAL OVERHEAD HAZARD. Use caution when working inside the cabinet. Keep the instrument side door closed over the Cleave Solution and Reset Buffer bottles.

Figure 11 Positions of reagent bottles on the side of the instrument.



6. Install the prepared Imaging Buffer, prepared 1X T4 Ligase Buffer, and prepared Universal Buffer into the appropriate positions in the chiller block (see Figure 12). Make sure that the tubes on the caps of the reagent bottles are fully screwed on.

Figure 12 Positions of buffer bottles in the chiller block.



Check the waste level

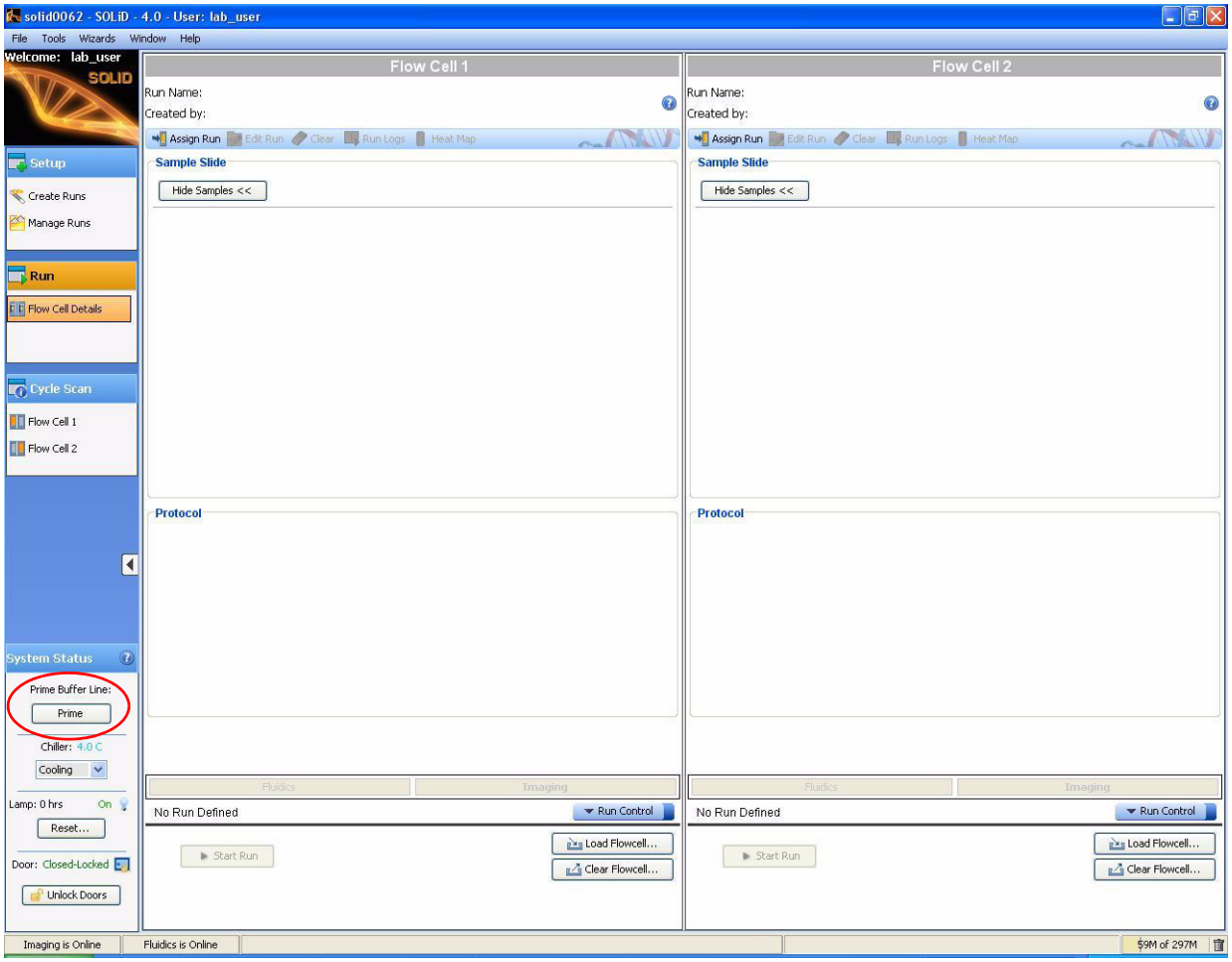
1. Check the level of waste in the 10-L carboy.
2. If the carboy is more than ¼ full, properly dispose of the waste according to your institution's environmental health and safety guidelines.

Prime Instrument and Storage Buffer lines

IMPORTANT! The priming of the lines must be performed prior to each run whether the Instrument and/or Storage Buffers were changed or not.

1. Under the System Status menu, click **Prime** (see Figure 13).

Figure 13 Click **Prime** to prime the instrument and storage buffer lines.



2. During priming, open the middle front door of the SOLiD® 4 Analyzer to check that the syringe is filled with buffer when the plunger is at the aspiration stage (see Figure 14). Confirm that the buffer lines are filled with buffer. If the syringe is not filled with buffer even at the last stroke, click **Prime** again.

Figure 14 Syringe on the SOLiD® 4 Analyzer.

Install slide(s) on the instrument

Prepare the slide

IMPORTANT! Before removing the slide from the SOLiD® Deposition Chamber, ensure that either the instrument flowcell is ready or a SOLiD® Slide Storage Chamber is available.

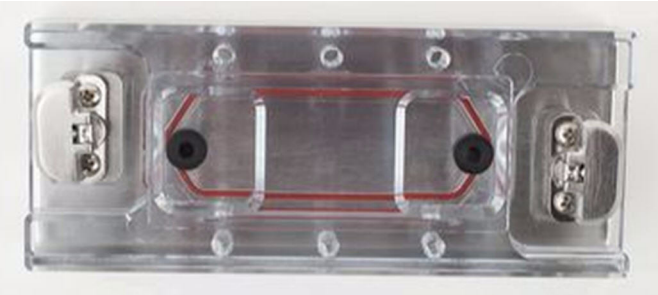
1. Remove the 3-mm adhesive disks.
2. Pour enough SOLiD® XD Slide Deposition Buffer v2 to cover the top of the SOLiD® Deposition Chamber (1.5 mL in each port for the 1-Well SOLiD® Deposition Chamber and 6 mL for the 4-Well and 8-Well SOLiD® Deposition Chambers).
3. Using an appropriate pipettor and pipette tip, press down the plunger button, then place the tip into one of the portholes of the well. Slowly release the pipettor plunger button, then aspirate the SOLiD® XD Slide Deposition Buffer v2. Fresh SOLiD® XD Slide Deposition Buffer v2 is drawn into the well to replace the old Deposition Buffer. Repeat this procedure for the other wells.
4. Gently loosen the SOLiD® Deposition Chamber screws. As the screws are loosened, more fresh SOLiD® XD Slide Deposition Buffer v2 is drawn into the Deposition Chamber.
5. Open the SOLiD® Deposition Chamber lid, then carefully remove the SOLiD® 4 or Opti Slide Carrier assembly from the Deposition Chamber.
6. Immediately pour Slide Storage Buffer over the slide to completely cover the beads. Allow the Slide Storage Buffer to flow off onto a paper towel or into a waste container.
7. Repeat step 6.

8. Immediately place the SOLiD® 4 or Opti Slide Carrier assembly onto the instrument or into the SOLiD® Slide Storage Chamber.

IMPORTANT! The Deposition Chamber should not have any contact with Slide Storage Buffer or Overlay Buffer (from previous versions of SOLiD® instruments).

STOPPING POINT If you are storing the slide, place the SOLiD® 4 or Opti Slide Carrier assembly into the SOLiD® Slide Storage Chamber, then fill with 5 mL Slide Storage Buffer. Store the slide at 4°C until the slide is ready for use (see Figure 15).

Figure 15 SOLiD® Slide Storage Chamber.

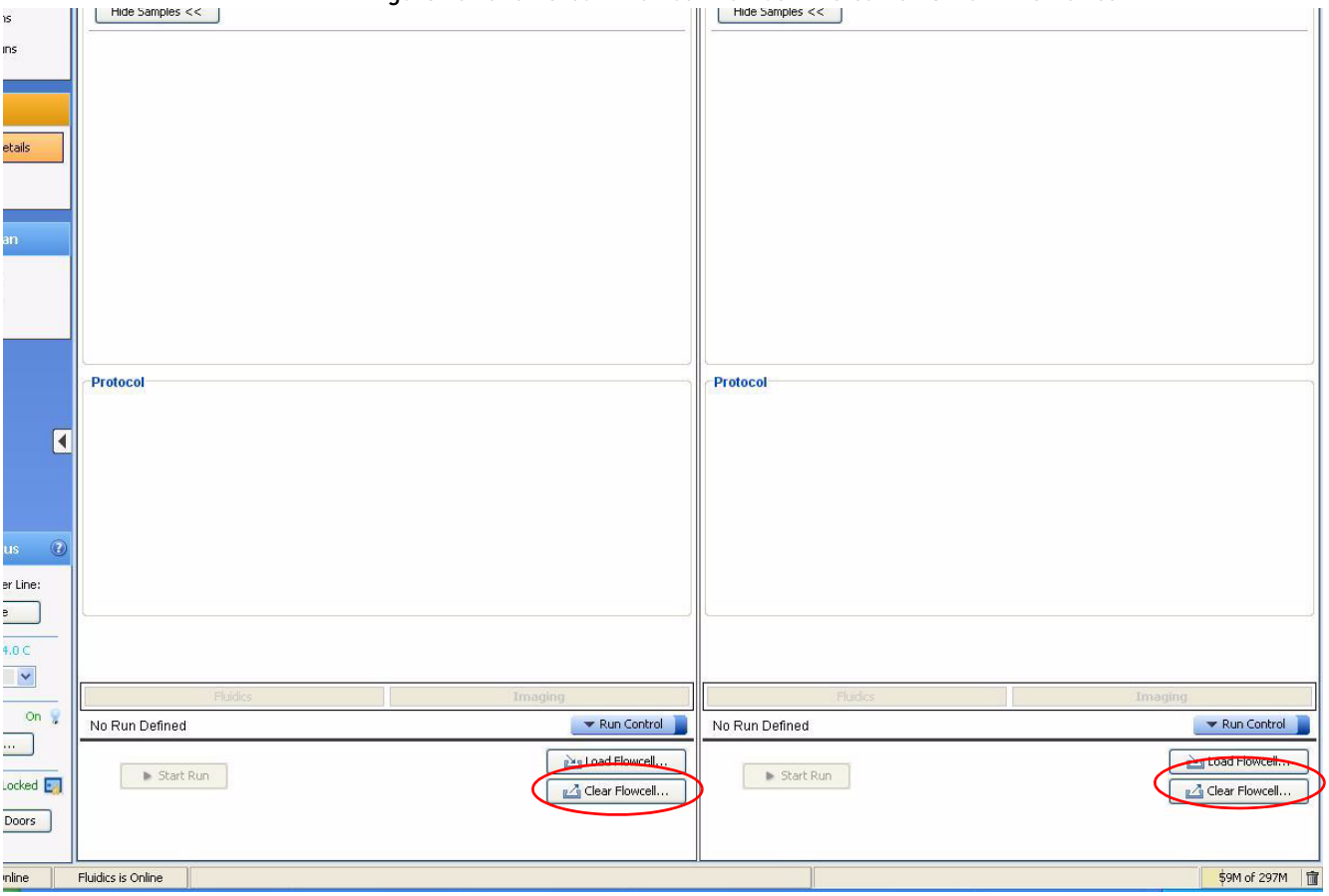


Install the slide

IMPORTANT! Before removing a slide from a previous run, ensure that the run, images, and data collected from the previous run are satisfactory. For more information, refer to the *Applied Biosystems SOLiD® 4 System SETS Software User Guide* (PN 4448411).

1. Check to see if a SOLiD® 4 or Opti Slide Carrier assembly from a previous run is present in the flowcell chamber. If a SOLiD® 4 or Opti Slide Carrier assembly *is* present in the flowcell chamber, proceed with steps 2 to 4. If a SOLiD® 4 or Opti Slide Carrier assembly is *not* present in the flowcell chamber, skip to step 5.
2. For each flowcell to be used, click the **Clear Flowcell** button at the bottom of the flowcell panel (see Figure 16).

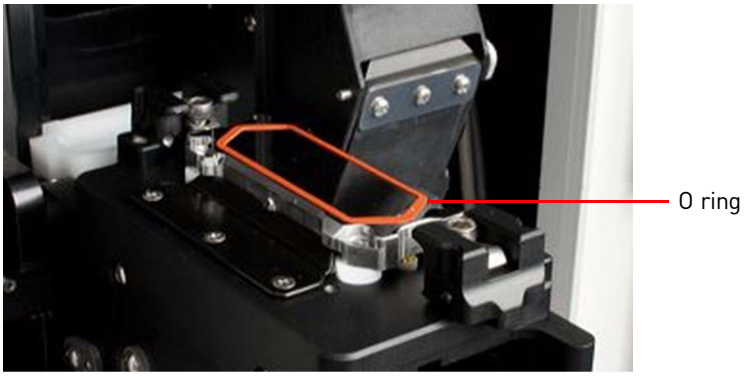
Figure 16 Click **Clear Flowcell** to flush the contents from the flowcell.



3. After clearing each flowcell, open the appropriate flowcell chamber.
4. Remove the SOLiD® 4 or Opti Slide Carrier assembly from the previous run. If the slide will be reused, place the SOLiD® 4 or Opti Slide Carrier assembly into a SOLiD® Slide Storage Chamber, then fill the chamber with Slide Storage Buffer. Store the SOLiD® Slide Storage Chamber at 4°C.
5. Clean the flowcell block with 70% ethanol and Kimwipes® wipers to remove residue.

6. Inspect the O-ring and reseat it if necessary (see Figure 17; for details, see Appendix B, "Install the SOLiD® System Flowcell O-ring" on page 102). Check the O-ring for cuts and abrasions. If any abnormalities are observed, replace it. Inspect the O-ring grooves for debris or contamination and clean with water as needed.

Figure 17 O-ring installed on flowcell.



7. Ensure that the instrument and storage buffer lines are primed before loading a slide, or the slide will dry out.

8. Insert the SOLiD® 4 or Opti Slide Carrier onto the instrument. Work quickly to prevent the slide from drying out.

   a. Remove the SOLiD® 4 or Opti Slide Carrier assembly from the SOLiD® Deposition Chamber or from the SOLiD® Slide Storage Chamber.

   b. Place the SOLiD® 4 or Opti Slide Carrier assembly into the open flowcell, engaging the alignment key on the carrier with the corresponding part on the flowcell.

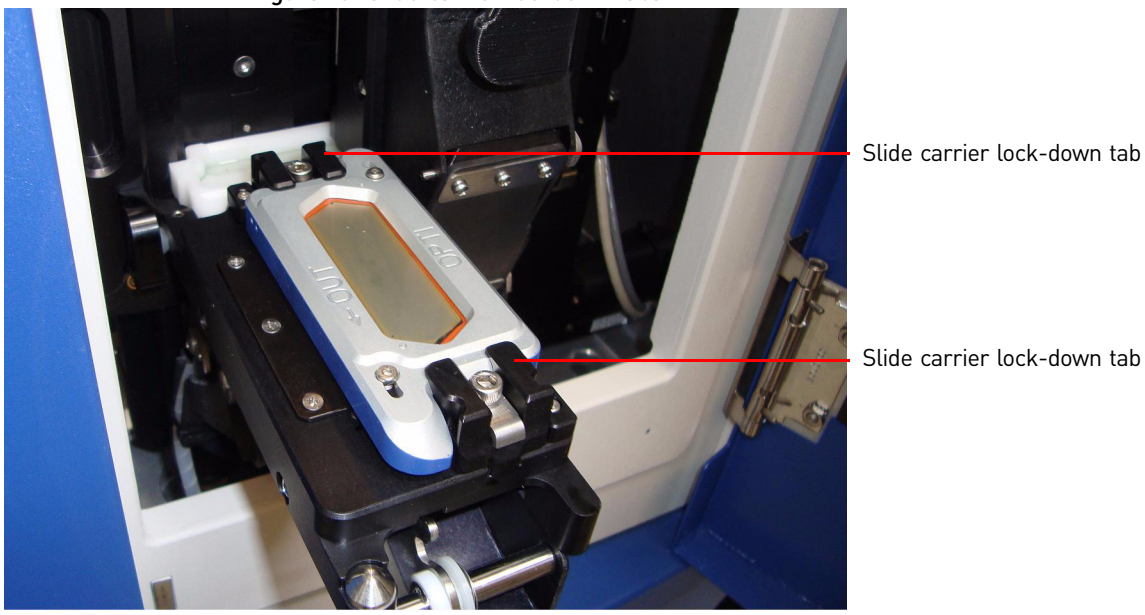
   c. Slide the two slide carrier lock-down tabs on the flowcell inward until they are positioned over and flush with the carrier (see Figure 18).

      IMPORTANT! Ensure that the tabs are flush with the carrier. If necessary, loosen the Allen screws further, then slide the tabs over the slide carrier.

   d. To properly seat the carrier on the flowcell, gradually tighten the 2 Allen screws on both lock-down tabs in an alternating fashion to 20 inch-pounds.

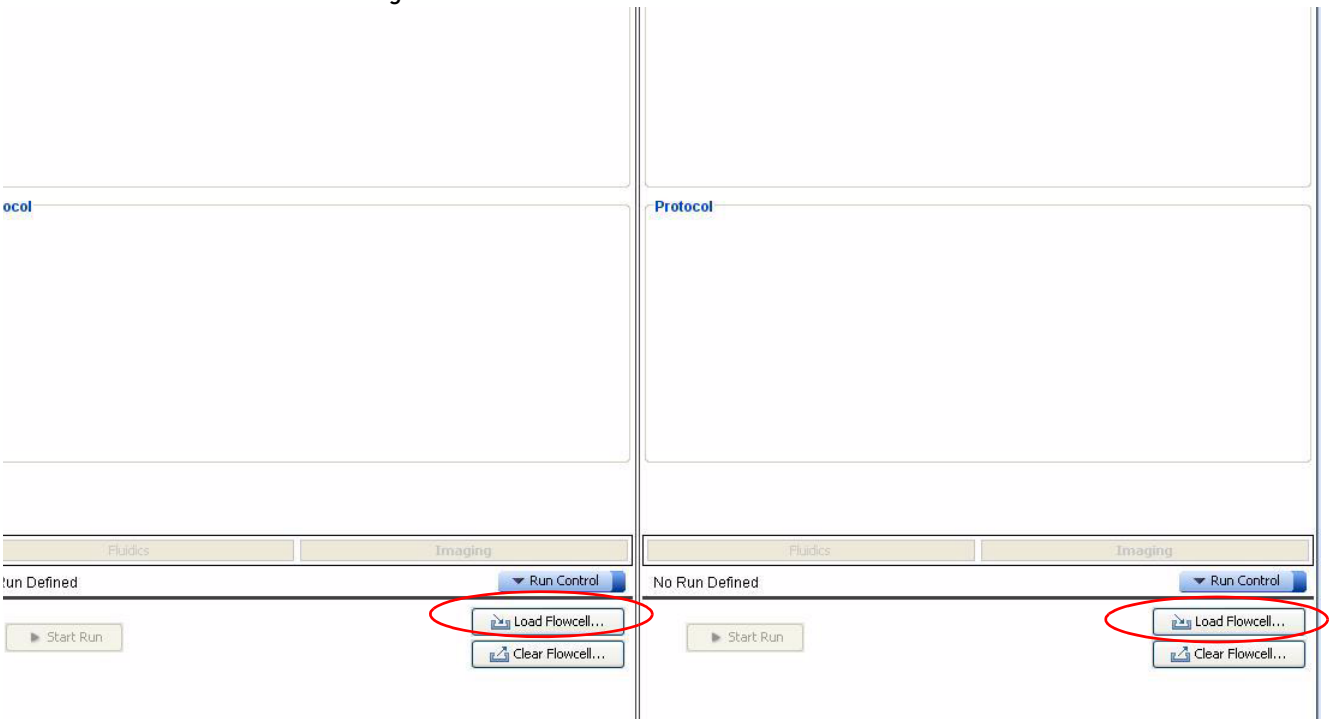
   e. Rotate the flowcell up and lock it into the scan position.

Figure 18 Slide carrier lockdown tabs.



9. Close the instrument doors.
10. Click the **Load Flowcells** button located at the bottom of the flowcell panel. Each flowcell has its own Load Flowcells button (see Figure 19).

Figure 19 Click **Load Flowcells** to load the flowcell with buffer.



11. Click **Yes** to start loading the flowcell.

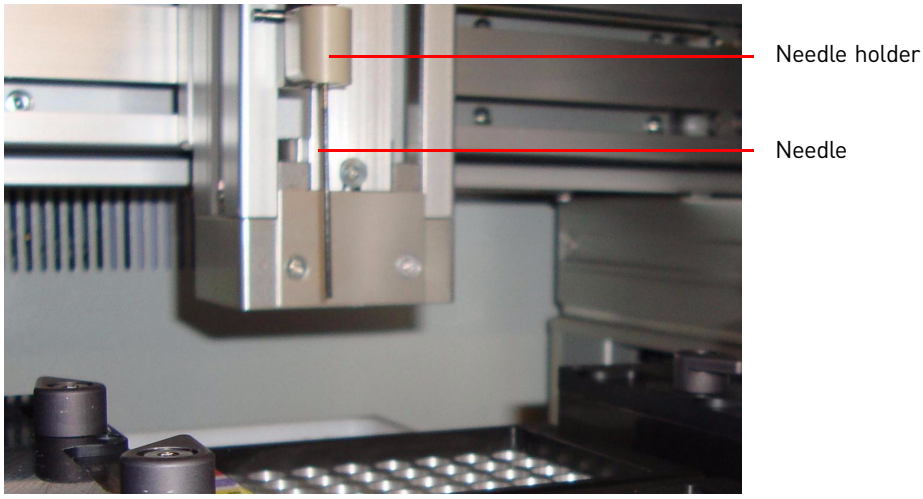
IMPORTANT! The flowcell should be loaded within 1–2 minutes of slide installation. Slides should be installed one at a time, with flowcells loaded before installing a second slide.

12. Check for leaks to ensure that the slide does not dry out.
13. If only one flowcell will be used, ensure that unused flowcell slide carrier lock-down tabs are pushed all the way in and tightened before closing the unused flowcell.

Install reagent strip(s) on the instrument

1. Clean the needle and bottom of the needle holder with a Kimwipes® wiper (see Figure 20). If needed, first moisten the Kimwipes® wiper with deionized water or use a pre-moistened alcohol pad.

Figure 20 Needle and needle holder.



2. Thaw the appropriate reagent strip(s) on ice.

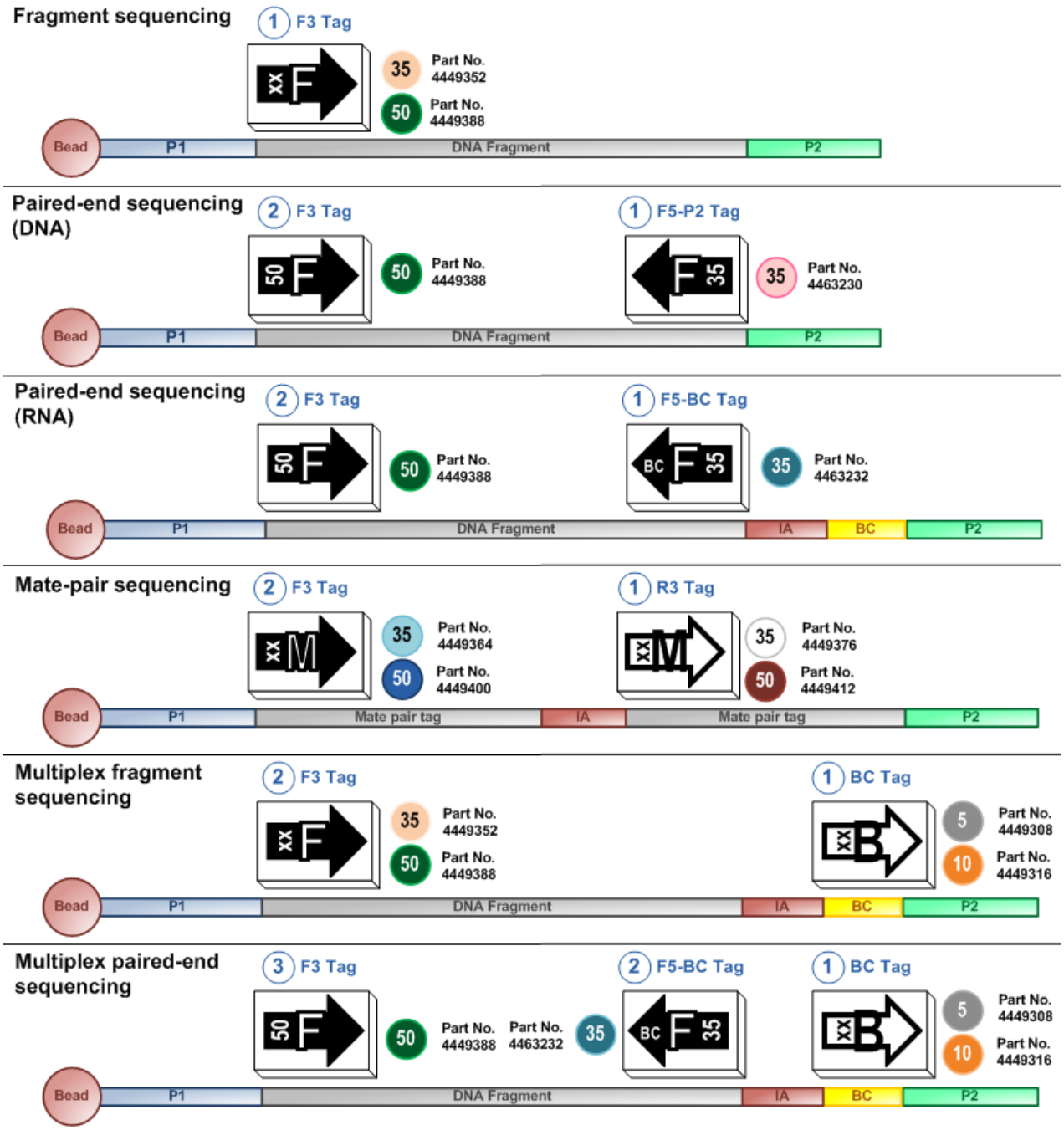
   See Figure 21 to determine which reagent strips should be used for a sequencing run. Use reagent strips from the boxes that match the arrow symbol for the appropriate library and tag.

IMPORTANT! Verify that there is sufficient volume of Reset Buffer for each tag sequenced. The SOLiD® ToP Instrument Buffer contains sufficient Reset Buffer to reset two tags (see Appendix C, "On-Instrument Reagent Volumes and Reagent Strip Layouts" on page 121). For multiplex sequencing, a bottle of Reset Buffer is included with multiplex sequencing kits to account for sequencing the BC tag.

The blue circled numbers indicate the order in which the tags should be sequenced for each library type. The XX represents read length options in bases (35 and 50 for F3 and R3 tags; 5 and 10 for BC tag).

Note: The 5-bp barcode sequencing reagents can be used if only barcodes 1–16 are being used. The 10-bp barcode sequencing reagents are needed for any set that includes barcodes 17–96. For example, if you are sequencing 32 barcoded fragment libraries using barcodes 1–32, you should use the 10-bp barcode sequencing reagents. Only 5-bp or 10-bp barcode reads (not both) may be sequenced on a single slide.

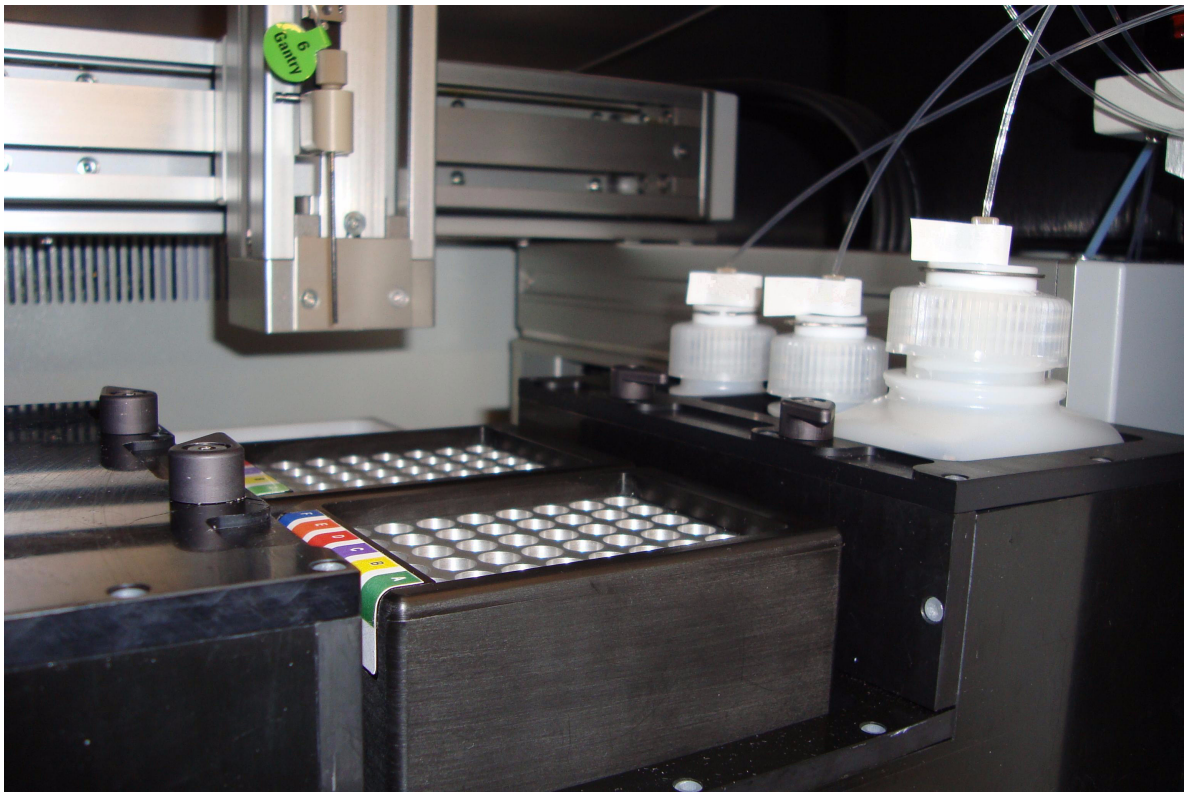
Figure 21 Reagent strip box symbols corresponding to fragment sequencing, paired-end sequencing, mate-pair sequencing, multiplex fragment sequencing, and multiplex paired-end sequencing.



3. Place the reagent strip(s) in an ABgene® 96-well square-well storage plate and centrifuge at 160 × g for 2 minutes.

4. Verify that the reagent strip blocks are oriented and seated properly in the chiller block. The block must engage the orientation key to fit properly. The orientation key (cut corner) is in the upper-left position when the block is placed on the instrument (see Figure 22).

Figure 22 The Reagent Strip Block.



IMPORTANT! Ensure that the chiller block temperature is below 10°C before proceeding with reagent strip installation. Under the System Status menu, select **Cooling** from the Chiller drop-down menu (see Figure 9 on page 26).

5. When the temperature is less than 10°C, place the reagents in the appropriate location in the chiller block (see Table 6 and Figure 26 on page 41).

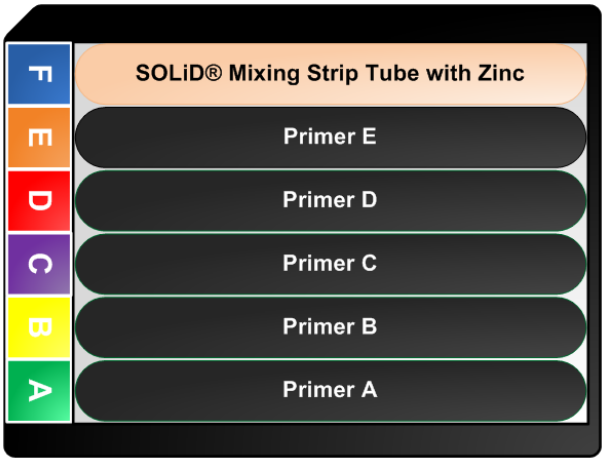
   See Figures 23 to 25 for the appropriate location of reagent strips on the chiller block. For information about the contents of the reagent strip tubes, see Appendix C, "Reagent strip layouts" on page 124.

Table 6 Where to place the strip tubes

If using flowcell...	Then place the strip tube(s) in the...
1	Front block
2	Rear block

Figure 23 Reagent strip block layout for WFA.

Figure 24 Reagent strip block layouts for sequencing F3 and R3 tags.



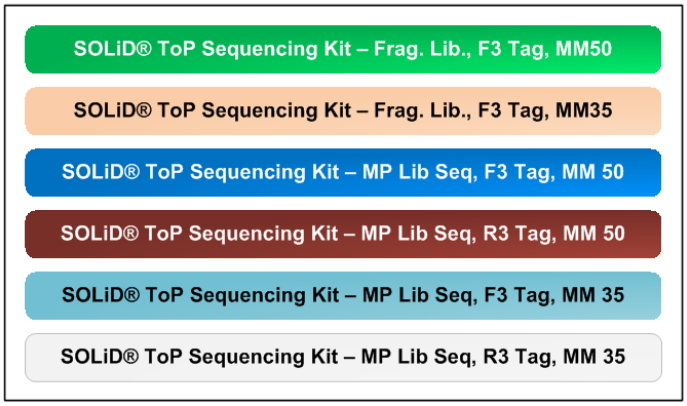

SOLiD® ToP Sequencing Kit – Frag. Lib., F3 Tag, MM50
SOLiD® ToP Sequencing Kit – Frag. Lib., F3 Tag, MM35
SOLiD® ToP Sequencing Kit – MP Lib Seq, F3 Tag, MM 50
SOLiD® ToP Sequencing Kit – MP Lib Seq, R3 Tag, MM 50
SOLiD® ToP Sequencing Kit – MP Lib Seq, F3 Tag, MM 35
SOLiD® ToP Sequencing Kit – MP Lib Seq, R3 Tag, MM 35

Figure 25 Reagent strip block layouts for sequencing F5-P2 and F5-BC tags.

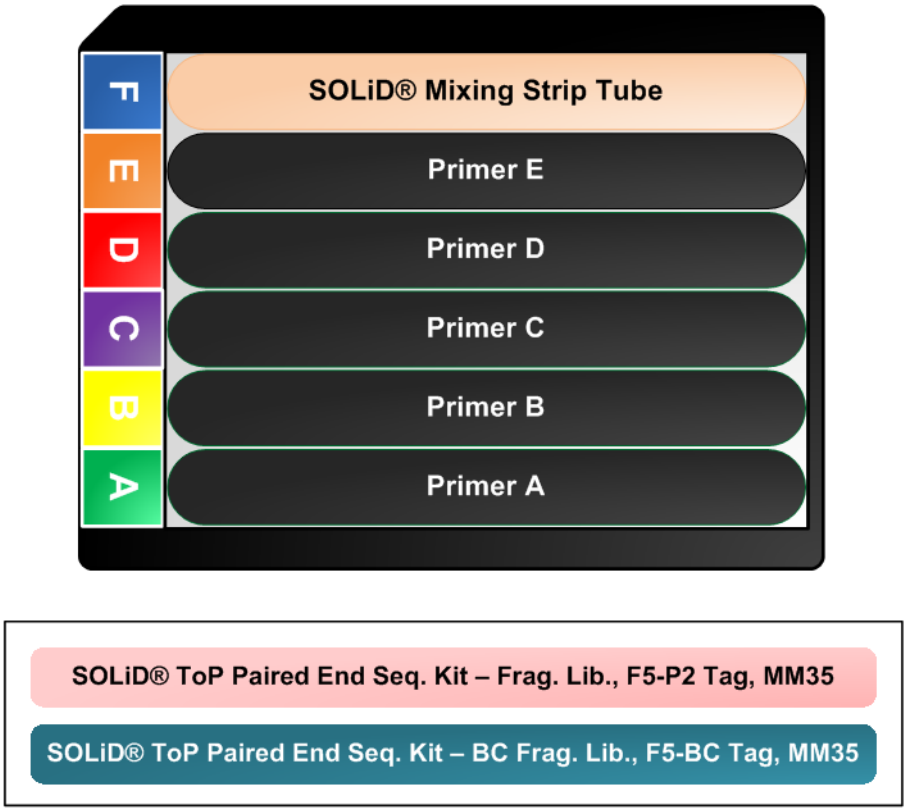


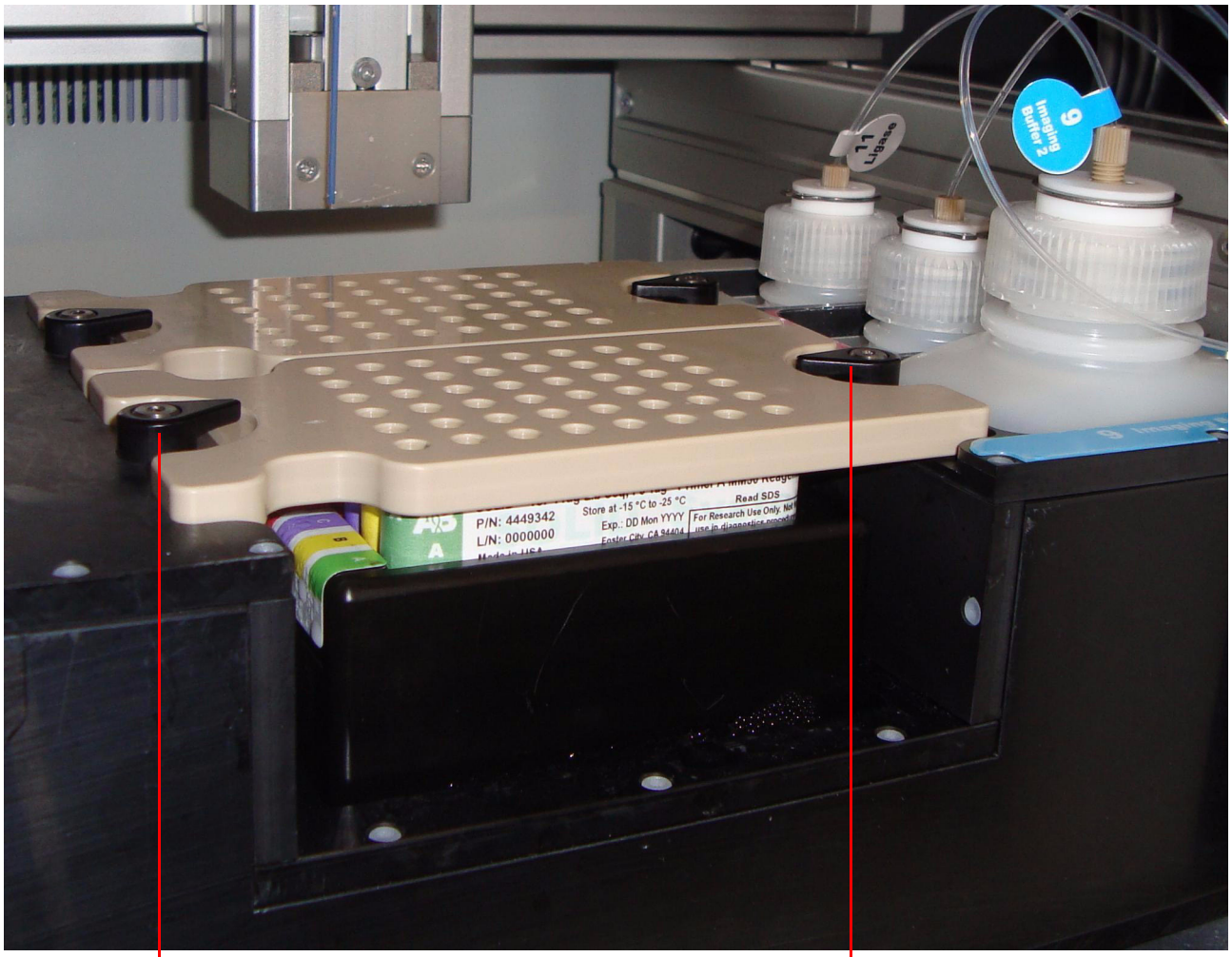
Figure 26 Position of the sequencing reagent blocks for flowcells 1 and 2.

11 Ligase
9 Ligation Buffer 2
SOLiD™ ToP Frag Lib Seq, F3 Tag - Primer A MM50 Reagent
P/N: 4449342
L/N: 0000000
Store at -15 °C to -25 °C
Read SDS
Exp.: DD Mon YYYY
For Research Use Only

6. Place the cover over the reagent strips and secure them using the cover fasteners (see Figure 27).

   Note: Ensure that the top and bottom of the cover is free of splattered wet or dry reagents (see Appendix B, "Clean the reagent strip cover" on page 103).

Figure 27 Securely fastened reagent strip covers.



3 Set Up, Control, and Monitor the Run

This chapter covers:

Section 3.1 Set up and perform a workflow analysis (WFA) run. 44

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- Workflow 44
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- View the WFA report 53

Section 3.2 Set up and perform a sequencing run 56

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- Create a sequencing (non-multiplex) run record. 58
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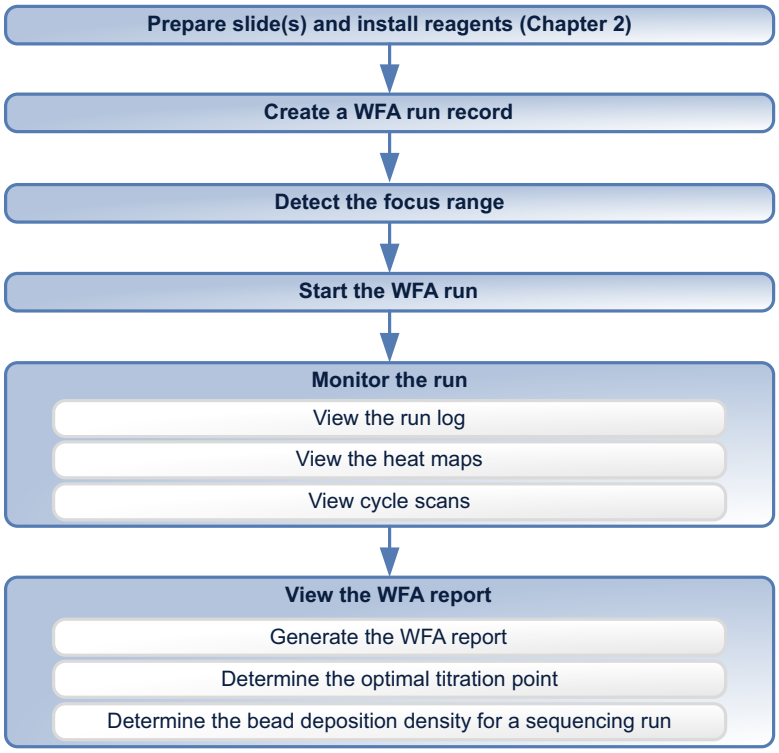
Section 3.1 Set up and perform a workflow analysis (WFA) run

Materials and equipment required

See Appendix A on page 83 for a list of equipment, kits, and consumables necessary to set up a workflow analysis (WFA) run.

Workflow


Prepare slide(s) and install reagents (Chapter 2)
Create a WFA run record
Detect the focus range
Start the WFA run
Monitor the run
View the run log
View the heat maps
View cycle scans
View the WFA report
Generate the WFA report
Determine the optimal titration point
Determine the bead deposition density for a sequencing run

Workflow overview

Create a WFA run record

A WFA run record is created using the SOLiD® Instrument Control Software (ICS).

Detect the focus range

Two methods exist for determining the focus range: automatic and manual. You should first attempt automatic range detection. If automatic detection fails, use the manual mode.

Start the WFA run

Start the run by using the SOLiD® Instrument Control Software. Before starting a run, you may need to remove previous data may need to ensure maximum disk space for new results.

Monitor the run

While executing a WFA run, the instrument performs a Prescan. In a Prescan, all of the beads are labeled and their positions on the slide are recorded to derive a focal map and then they undergo a single ligation cycle. The run can be monitored through the ICS or the SETS browser by using the Run Log, Heat Map, and Cycle Scans.

Each flowcell generates a separate Run Log that records high-level events, such as fluidic modules and slide scanning. The start and stop times of these events, as well as any pauses or errors during the run, are also recorded. The Run Log is particularly useful in helping you anticipate and schedule reagent refills or troubleshoot instrument errors.

Heat maps are generated from the analysis of the focal map images and analysis of each ligation cycle. A heat map is a colorized display of a particular metric (*bead count*, *bead signal*, or *image signal*) across all the panels for a run (for definitions of the metrics, see the "Glossary" on page 165).

For each flowcell, the corresponding Cycle Scans window provides nearly real-time feedback on initial data quality on a per-cycle basis.

View the WFA report

You can view a WFA report in the SOLiD® Experiment Tracking System (SETS) after the run is complete. Three important metrics are generated in the WFA report: *P2#/P1# ratio*, *On-Axis beads*, and *Titration Metric*. These metrics guide the selection of the best-performing bead population based on different titration points used in ePCR (for definitions of the metrics, see the "Glossary" on page 165). In general, the closer the image data points are on-axis, the higher the quality of data obtained due to good bead deposition and chemistry.

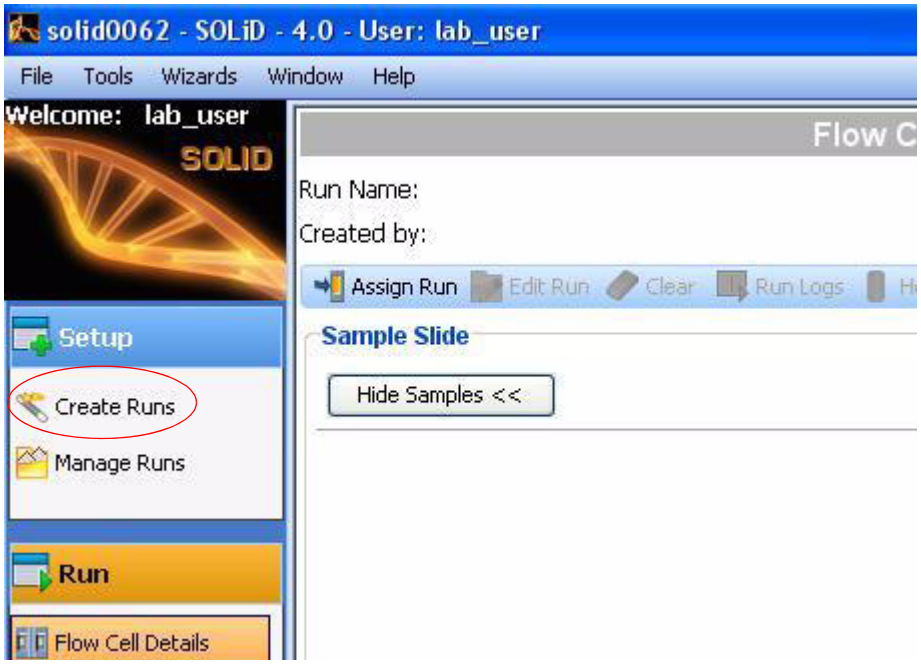
From the WFA data, you can also estimate the bead deposition density to be used when preparing a slide for sequencing. Differences between bead concentration measured on the NanoDrop® ND-1000 Spectrophotometer and the concentration actually detected by the bead counting algorithm on the instrument may occur. In order to maximize throughput in a SOLiD® System sequencing run, a bead density of 300,000 P2-positive beads per panel is recommended.

Create a WFA run record

There are two ways to create a WFA run. The first method is to use the Run wizard (see below). The second method is to import a .txt file that contains the run definition (see "Set up a run by importing a Run Definition file" on page 105). Importing a .txt file saves time re-entering information of a repeated run. The .txt file can be generated on an off-instrument computer.

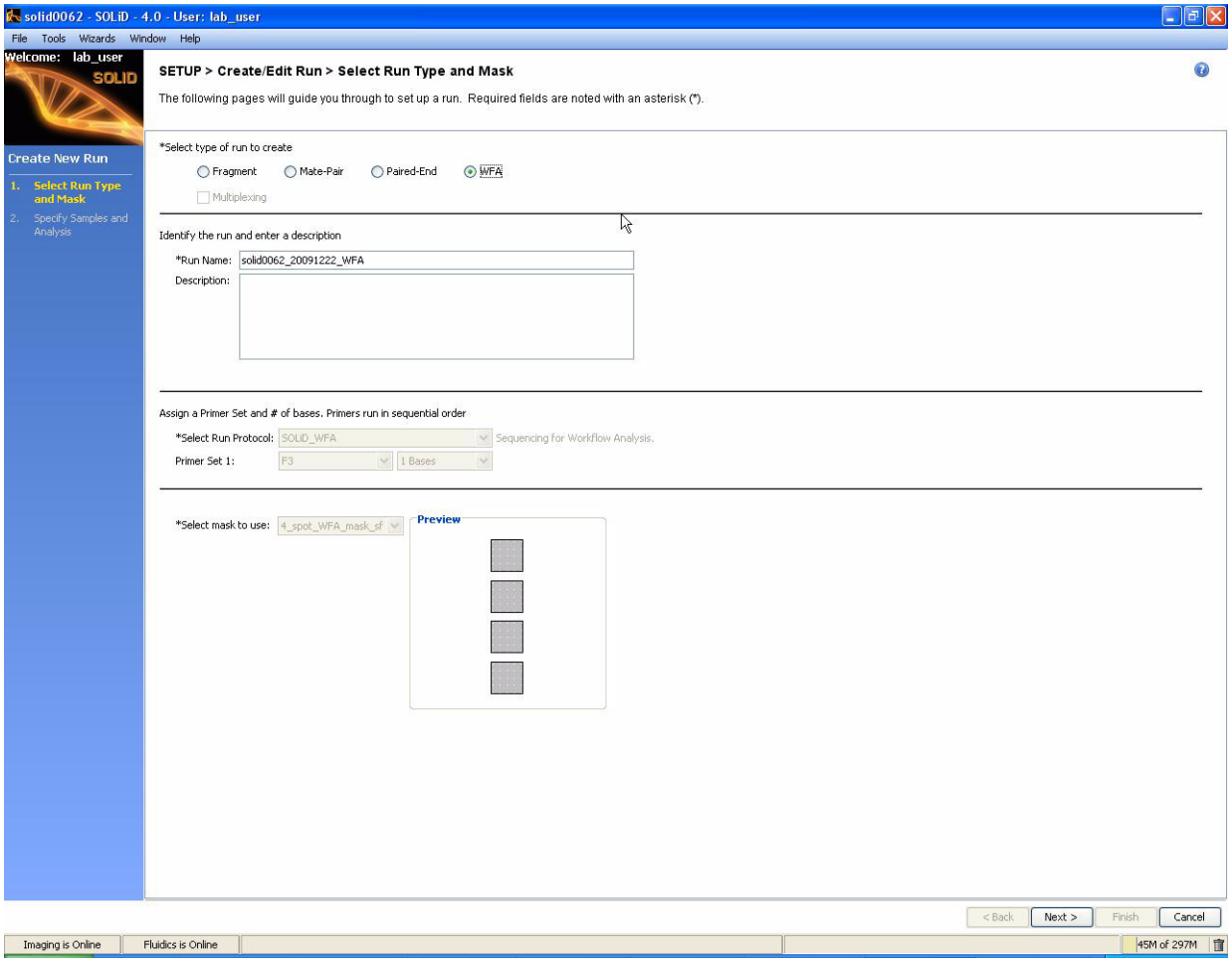
1. Click **Create Runs** in the Setup task pane on the left menu pane of the ICS (see Figure 28 on page 46).

   Figure 28 Use the Run wizard to create a WFA run.



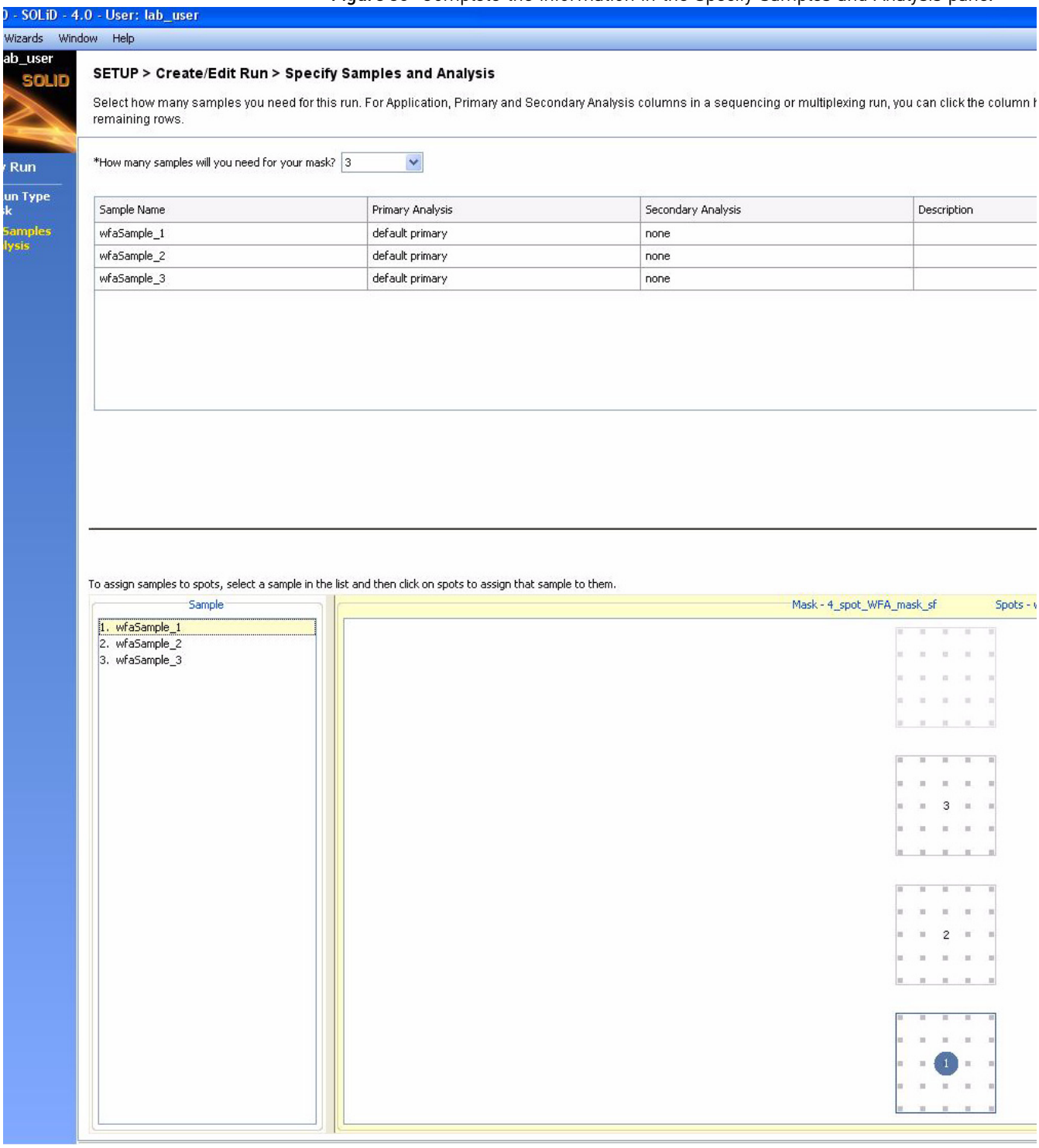
2. Complete the information in the Select Run Type and Mask pane (see Figure 29 on page 47):
   a. Select the **WFA** option.
   b. (Optional) Type a new run name.
   c. (Optional) Enter a description.
   d. Ensure that the mask **4_spot_WFA_mask_sf** is selected.
   e. Click **Next**.

Figure 29 Complete the information in the Select Run Type and Mask pane.


solid0062 - SOLiD - 4.0 - User: lab_user
File Tools Wizards Window Help
Welcome: lab_user
SOLiD
Create New Run
1. Select Run Type and Mask
2. Specify Samples and Analysis
SETUP > Create/Edit Run > Select Run Type and Mask
The following pages will guide you through to set up a run. Required fields are noted with an asterisk (*).
*Select type of run to create
Fragment
Mate-Pair
Paired-End
WFA
Multiplexing
Identify the run and enter a description
*Run Name: solid0062_20091222_WFA
Description:
Assign a Primer Set and # of bases. Primers run in sequential order
*Select Run Protocol: SOLiD_WFA
Sequencing for Workflow Analysis.
Primer Set 1: F3
1 Bases
*Select mask to use: 4_spot_WFA_mask_sf
Preview
< Back
Next >
Finish
Cancel
Imaging is Online
Fluidics is Online
45M of 297M

3. Complete the information in the Specify Samples and Analysis pane (see Figure 30):
   a. Assign samples to spots.
   b. Click **Finish**.

Figure 30 Complete the information in the Specify Samples and Analysis pane.



4. Choose either to assign a run to a flowcell for immediate use or to store the run in the instrument database for later use, then click **OK**.
5. To assign a run previously saved to the database:
   a. Click on **Manage Runs** in the task pane.
   b. Click the run, then select **Assign to Flowcell**.

c. Choose a flowcell, then click **OK**.

6. (Optional) Repeat the above steps for the other flowcell if performing a WFA run on a second slide.

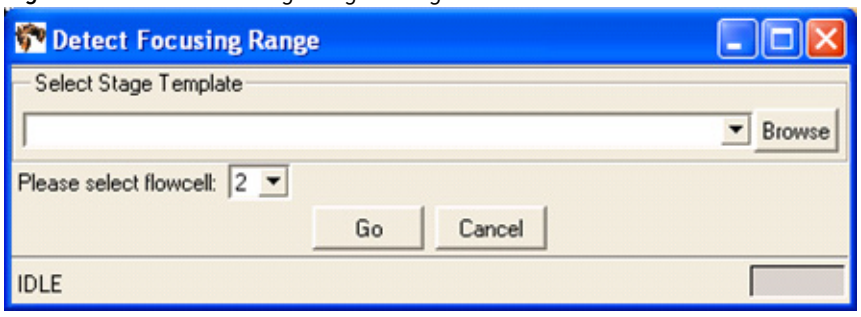
Detect the focus range

Note: If automatic focus range detection fails, determine the focus range manually (see "Manually find the focus range" on page 108).

1. Close the front doors of the SOLiD® 4 Analyzer. Open the Imager window by choosing **Windows** ▸ **Imaging System**.
2. Select **Tools** ▸ **Detect Focusing Range**.
3. In the focusing range dialog, specify the stage template file by entering the name of the stage template file directly or by clicking the **Browse** button to navigate to a suitable stage template file (see Figure 31).

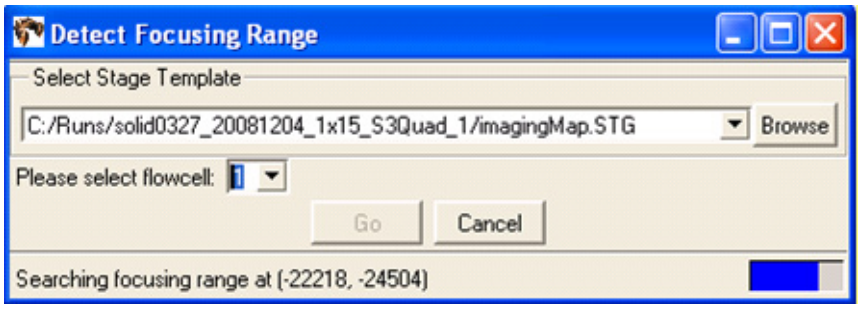
   The stage template must match the slide in the target flowcell(s). If you created a run from the ICS, then select the file **imagingMap.STG** (from the subdirectory in **C:\Runs**) whose name matches the name of the run (for example, select **C:\Runs\Solid0327_20081209_2_Oct_Test\imagingMap.STG**).

Figure 31 Detect Focusing Range dialog.



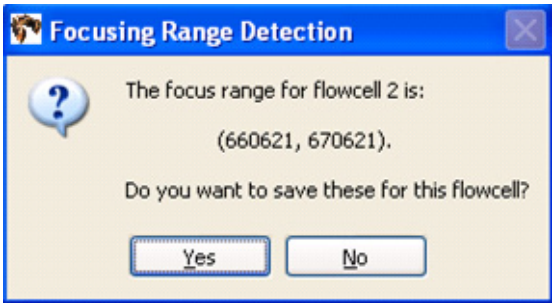
4. Select the flowcell using the drop-down menu, then press **Go**. The Imager works for several minutes while it determines the range. The blue progress bar indicates how close it is to completion (see Figure 32). You can also click **Cancel** so that the Imager aborts the ranging operation.

Figure 32 Detect Focusing Range Dialog while detection in progress.



5. When the Imager is done, a dialog appears (see Figure 33). Click **Yes** if you want to replace the values in the local settings file. Click **No** if you want the Imager to discard the newly calculated focus range.

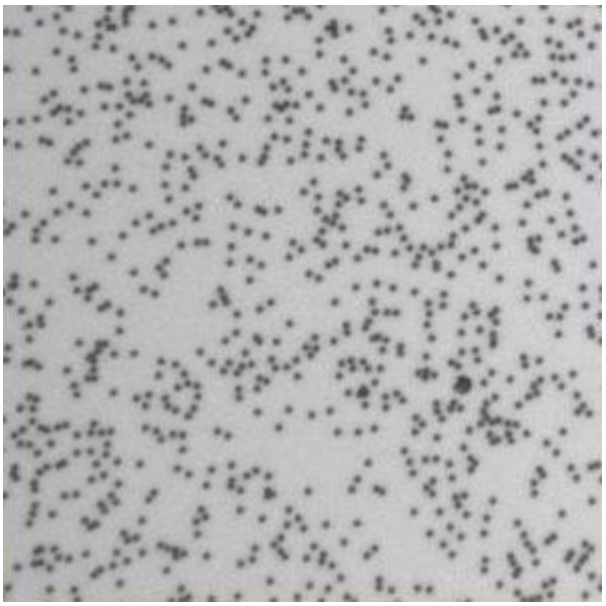
Figure 33 Confirm that you want to replace the local settings file.



6. Verify the validity of the newly calculated focus range by taking images at random locations.

IMPORTANT! You should see images of beads, ensuring that the algorithm was able to focus on the beads and not on other artifacts (see Figure 34). If you do not see bead images or if you see out-of-focus bead images, set the focus range manually (see "Manually find the focus range" on page 108).

Figure 34 Beads in focus.



Start the WFA run

Note: Before starting the run, make sure the air filter at the back of the instrument is not clogged with dust (see "Clean the air filter" on page 98).

1. Click **Start Run**.
2. If there is not enough room to store the data, the Start Run dialog appears (see Figure 35). Choose the appropriate option (see Table 7).

Figure 35 This Start Run dialog appears if there is not enough room to store data for the run.

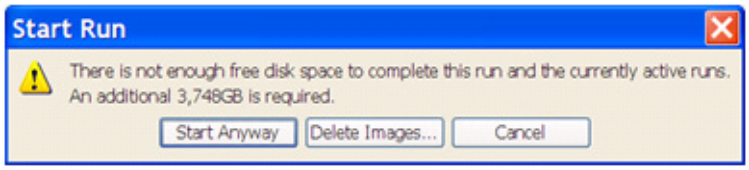

Start Run
There is not enough free disk space to complete this run and the currently active runs.
An additional 3,748GB is required.
Start Anyway
Delete Images...
Cancel

Table 7 Options for managing disk space

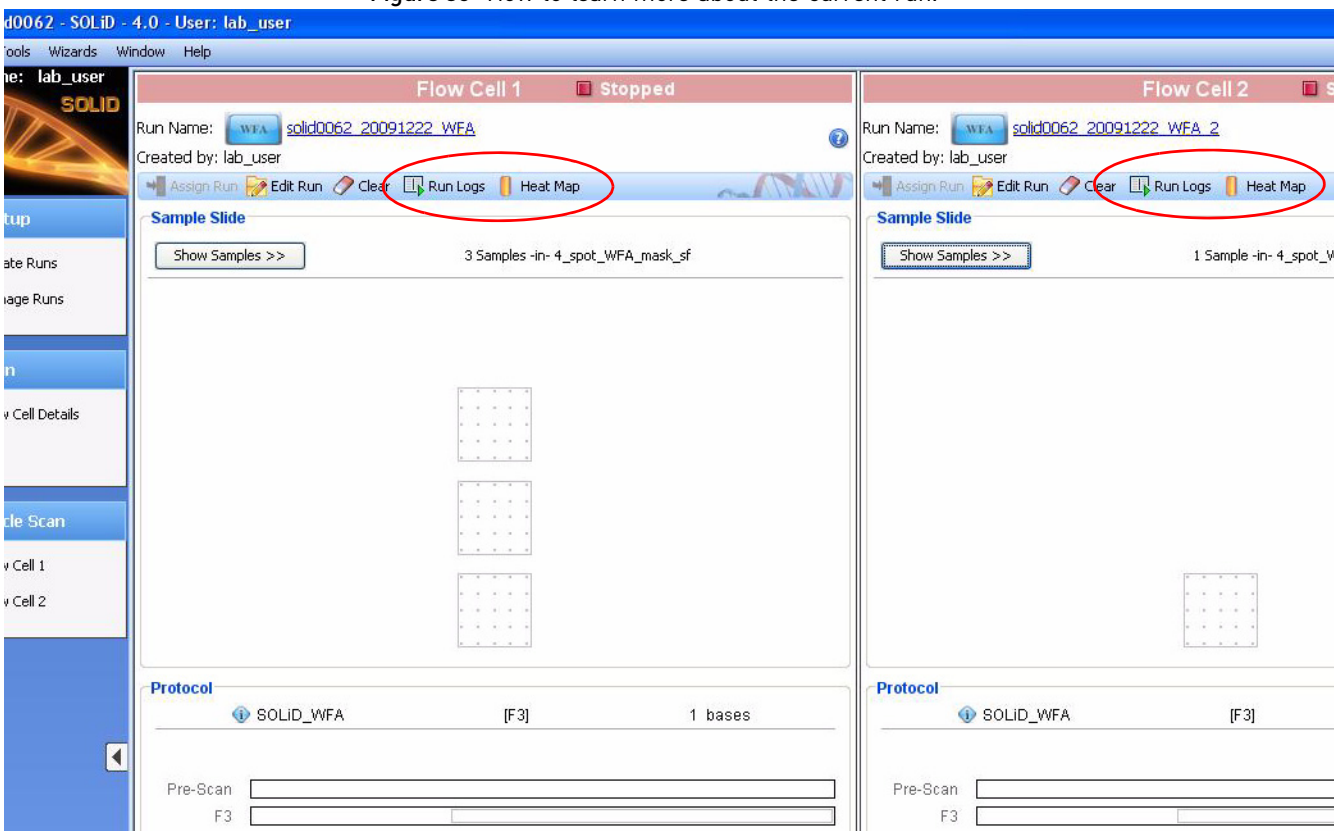
Option	Description
Start Anyway	Initiates the run. The instrument pauses itself when it runs out of free disk space.
Delete Images†	Launches Historical Runs page in SETS. Images and/or results can be deleted through SETS.
Cancel	Aborts the run.

† For more information on creating more available disk space, refer to the *Applied Biosystems SOLiD® 4 System SETS Software User Guide* (Part no. 4448411).

IMPORTANT! Before deleting any images, ensure that data analysis from the previous run is satisfactory and complete. For more information, refer to the *Applied Biosystems SOLiD® 4 System SETS Software User Guide* (Part no. 4448411).

3. After the run has been initiated, you can click the **Run Log**, **Cycle Scans**, and **Heat Map** buttons located at the top of the appropriate flowcell panel to learn more information about the current run (see Figure 36).

Figure 36 How to learn more about the current run.



IMPORTANT! Do not disturb the SOLiD® 4 Analyzer while in operation and do not open the flowcell during a pause in the run. Significant perturbations (for example, opening system parts, vibrations) during the run is detrimental to the results.

Monitor the WFA run

Note: To monitor the run remotely, use SETS from any computer to connect to the networked instrument [refer to the *Applied Biosystems SOLiD® 4 System SETS Software User Guide* (Part no. 4448411)]. If desired, set up e-mail notification regarding the instrument run and system information using SETS (refer to the *Applied Biosystems SOLiD® 4 System SETS Software User Guide*).

View the run log

1. Click **Run Log** located at the top right corner of the flowcell panel. A dialog box opens, describing a series of instrument events.
2. After you finish viewing the Run Log, click **Close**, located at the bottom of the Run Log window.

View the heat maps

1. To view the heat map showing bead densities found in the focal map images, click **Heat Map** located at the top right-hand corner of the flowcell panel (see Figure 36 on page 52).

2. Look for:
   - Uniform deposition of beads on the slide.
   - The actual average bead deposition density/panel value being similar in value to the targeted average bead deposition density/panel of 25,000 beads/panel for WFA run. A large number of missing panels could indicate a deposition problem.

   Note: The heat map may not immediately be available after the completion of the Prescan. The software must process all the images collected during the Prescan before the heat map is available. This process may take up to 30 minutes, depending on the number of panels imaged.
3. After you finish viewing the Heat Map, click **Close** located at the bottom of the Heat Map window.

View cycle scans

1. Select the appropriate flowcell (**Flow Cell 1** or **Flow Cell 2**) in the Cycle Scan menu on the task bar on the left.

   Note: Details regarding which parameters to monitor are described in the sequencing run section (see "Monitor the run" on page 77).
2. Click the heat map link to view the heat map for that cycle (see Figure 60 on page 81).
3. Left-click any square panel on the heat map to open the panel browser window. The panel browser allows you to view the focal map and the image for each fluorescent dye signal (see Figure 61 on page 82).
4. After you finish viewing the Cycle Scans, close the Cycle Scans window.

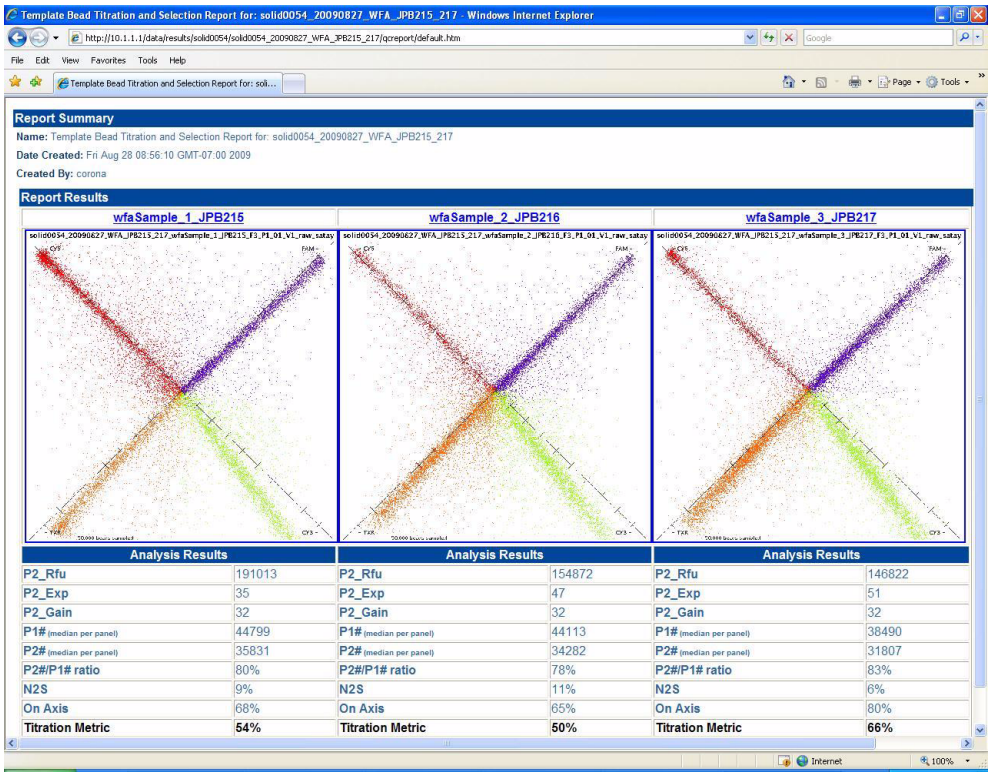
   Note: If the WFA run appears problematic, you can: (1) Allow the run to continue and troubleshoot after the run or (2) Pause the run and troubleshoot. Consult an Applied Biosystems SOLiD® Field Applications Specialist.

View the WFA report

Generate the WFA report

The WFA report is automatically generated and available in SETS when the WFA run finishes (see Figure 37). Refer to the *Applied Biosystems SOLiD® 4 System SETS Software User Guide* (Part no. 4448411).

Figure 37 One WFA report for a single 4-well slide displayed in SETS.



Determine the optimal titration point

Determine which titration has the highest titration metric. This titration is the optimal titration point.

Determine the bead deposition density for a sequencing run

1. Calculate the concentration of P2-positive beads using the formula below, where *X* is the volume of templated beads used for the WFA sample (equivalent to 15 million beads) and *P2#* is given by the WFA report:

$$\boldsymbol{X}\ \mu L = \frac{15 \times 10^6 \text{ beads}}{\#\ \text{beads}/\mu L\ (\text{according to NanoDrop}^{®})}$$

$$\boldsymbol{Y}\ \text{P2-positive beads}/\mu L = \frac{\text{P2\# beads/panel} \times 426\ \text{panels}}{\boldsymbol{X}\ \mu L}$$

Example:

For a sample with a concentration of 500,000 beads/µL measured by NanoDrop® ND-1000, where the WFA report indicates a P2# value of 20,000 beads/panel.

$$\boldsymbol{X}\ \mu L = \frac{15 \times 10^6 \text{ beads}}{500{,}000\ \text{beads}/\mu L} = 30\ \mu L$$

$$\boldsymbol{Y}\ \text{P2-positive beads}/\mu L = \frac{20{,}000\ \text{beads/panel} \times 426\ \text{panels}}{30\ \mu L}$$

$$= 284{,}000\ \text{P2-positive beads}/\mu L$$

2. Use the calculated concentration in place of the value determined by the NanoDrop® ND-1000 Spectrophotometer for more accurate deposition densities when preparing slides for sequencing. It is recommended that you use this resulting calculated bead concentration to determine the volume of beads for deposition.
3. Multiply that volume by 120% to calculate the volume of beads for deposition.

   Note: The 20% overage has been estimated empirically and is attributable to bead loss during washing and bead deposition steps. The overage factor may be adjusted by individual operators based on their experiences.

$$\boldsymbol{Z}\ \mu L\ \text{bead solution} = \frac{\text{Desired \# P2-positive beads to be deposited}}{\boldsymbol{Y}\ \text{P2-positive beads}/\mu L}$$

Example:

For a sample with a concentration of 284,000 P2-positive beads/µL to be deposited in one well of an 8-well SOLiD™ Deposition Chamber at a density of 300,000 beads/panel.

$$\boldsymbol{Z}\ \mu L\ \text{bead solution} = \frac{56\ \text{million P2-positive beads}}{284{,}000\ \text{P2-positive beads}/\mu L} \times 120\%$$

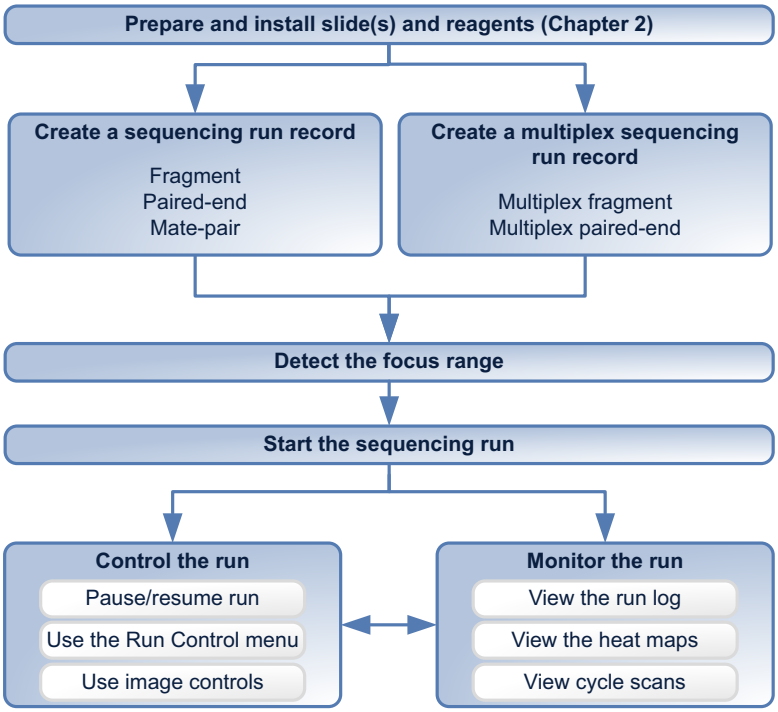
$$= 237\ \mu L\ \text{bead solution}$$

Section 3.2 Set up and perform a sequencing run

Materials and equipment required

See Appendix A on page 83 for a list of equipment, kits, and consumables necessary to set up a sequencing run.

Workflow



Workflow overview

Create a sequencing (non-multiplex) run record

A sequencing (non-multiplex) run record is created using the SOLiD® Instrument Control Software (ICS). Primary Analysis Settings and Secondary Analysis Settings should be created prior to creating a sequencing run record using SETS [refer to the *Applied Biosystems SOLiD® 4 System SETS Software User Guide* (Part no. 4448411)].

Create a multiplex sequencing run record

A multiplex sequencing run record is created using the SOLiD® Instrument Control Software (ICS). At this step, barcodes are matched to libraries. The run record file can be created using a spreadsheet program either on or off the instrument.

Primary Analysis Settings and Secondary Analysis Settings should be created prior to creating a sequencing run record using SETS [refer to the *Applied Biosystems SOLiD® 4 System SETS Software User Guide* (Part no. 4448411)].

Detect the focus range

Two methods exist for determining the focus range: automatic and manual. You should first attempt automatic range detection. If automatic detection fails, use the manual mode.

Start the sequencing run

The run is started using the SOLiD® Instrument Control Software. Before starting a run, previous data should be removed to ensure maximum disk space for new results. The instrument is limited to run 5 primers per flowcell. If you are performing a sequencing run with multiple tags, the instrument automatically pauses and stays paused until the reagents are replaced and the run is resumed.

Control the run

You can control how the SOLiD® 4 System collects sequencing data with the SOLiD® Instrument Control Software (ICS). With the ICS, you can repeat a primer to improve the real-time primary analysis results or set an early pause point to change reagents on your schedule. You can choose to turn off imaging of specific samples to collect only the best sequencing data.

To control the ICS, use the Run Control drop-down menu. Use the Run Control menu only if you understand clearly the series of fluidic and imaging steps in a run. Skipping or repeating certain steps could lead to errors in the resulting data. For example, with the ICS menus, you can repeat a ligation; however, you must first cleave the fluorescent label from the ligation product.

Monitor the run

While executing a sequencing run, the instrument performs a Prescan. In a Prescan, all of the beads are labeled and their positions on the slide are recorded to derive a focal map followed by a single ligation cycle. The run can be monitored using the Run Log, Heat Map, and Cycle Scans through the Instrument Control Software (ICS) or from the SETS browser.

Each flowcell generates a separate Run Log that records high-level events, such as fluidic modules and slide scanning. The start and stop times of these events and any pauses or errors that occur during the run, are also recorded. The Run Log is particularly useful in helping you anticipate and schedule reagent refills or troubleshoot instrument errors.

Heat maps are generated from the analysis of the focal map images and analysis of each ligation cycle. A heat map is a colorized display of a particular metric (*bead count*, *bead signal*, or *image signal*) across all the panels for a run (for definitions of the metrics, see the "Glossary" on page 165).

For each flowcell, the corresponding Cycle Scans window provides nearly real-time feedback on initial data quality on a per-cycle basis.

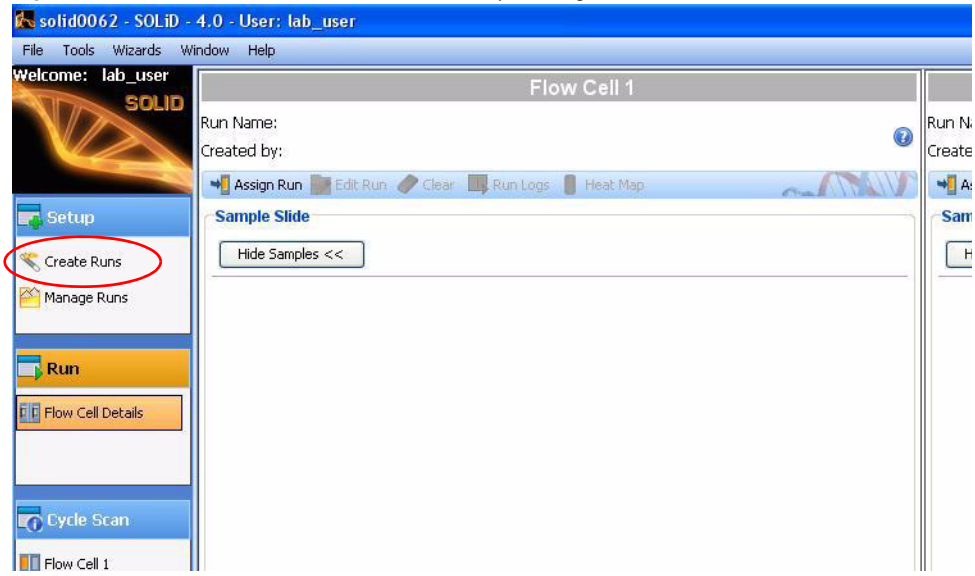
Create a sequencing (non-multiplex) run record

Note: For instructions to set up a multiplex sequencing run record, go to the next section "Create a multiplex sequencing run record" on page 61.

There are two ways to create a sequencing run. The first method is to use the Run wizard (see below). The second method is to import a .txt file that contains the run definition (see "Set up a run by importing a Run Definition file" on page 105). It saves time re-entering information of a repeated run. This file can be generated on an off-instrument computer.

1. Click **Create Runs** in the Setup task pane on the left menu pane of the ICS (see Figure 38).

Figure 38 Use the run wizard to create a sequencing run.



2. Complete the information in the Select Run Type and Mask pane (see Figure 39):
   a. Select the type of run:
      - If performing a fragment sequencing run, select **Fragment**
      - If performing a mate-pair sequencing run, select **Mate-Pair**
      - If performing a paired-end sequencing run, select **Paired-End**
   b. (Optional) Type a new run name.
   c. (Optional) Enter a description.
   d. Ensure that the Run Protocol is set to **SOLiD4**.
   e. Select **Primer Set 1** and **Primer Set 2**.
      - If performing a fragment sequencing run, leave **F3** as Primer Set 1.
      - If performing a mate-pair sequencing run, select **R3** as Primer Set 1 and **F3** for Primer Set 2.

IMPORTANT! The R3 tag must be run *first* in a mate-pair sequencing run. The reagent strips for the F3 tag do not contain Focal Map reagents.

- If performing a paired-end sequencing run, select **F5-P2** as Primer Set 1 and **F3** for Primer Set 2.
- If performing a paired-end sequencing run on a library prepared using the SOLiD™ Total RNA-Seq Kit, select **F5-BC** as Primer Set 1 and **F3** for Primer Set 2.

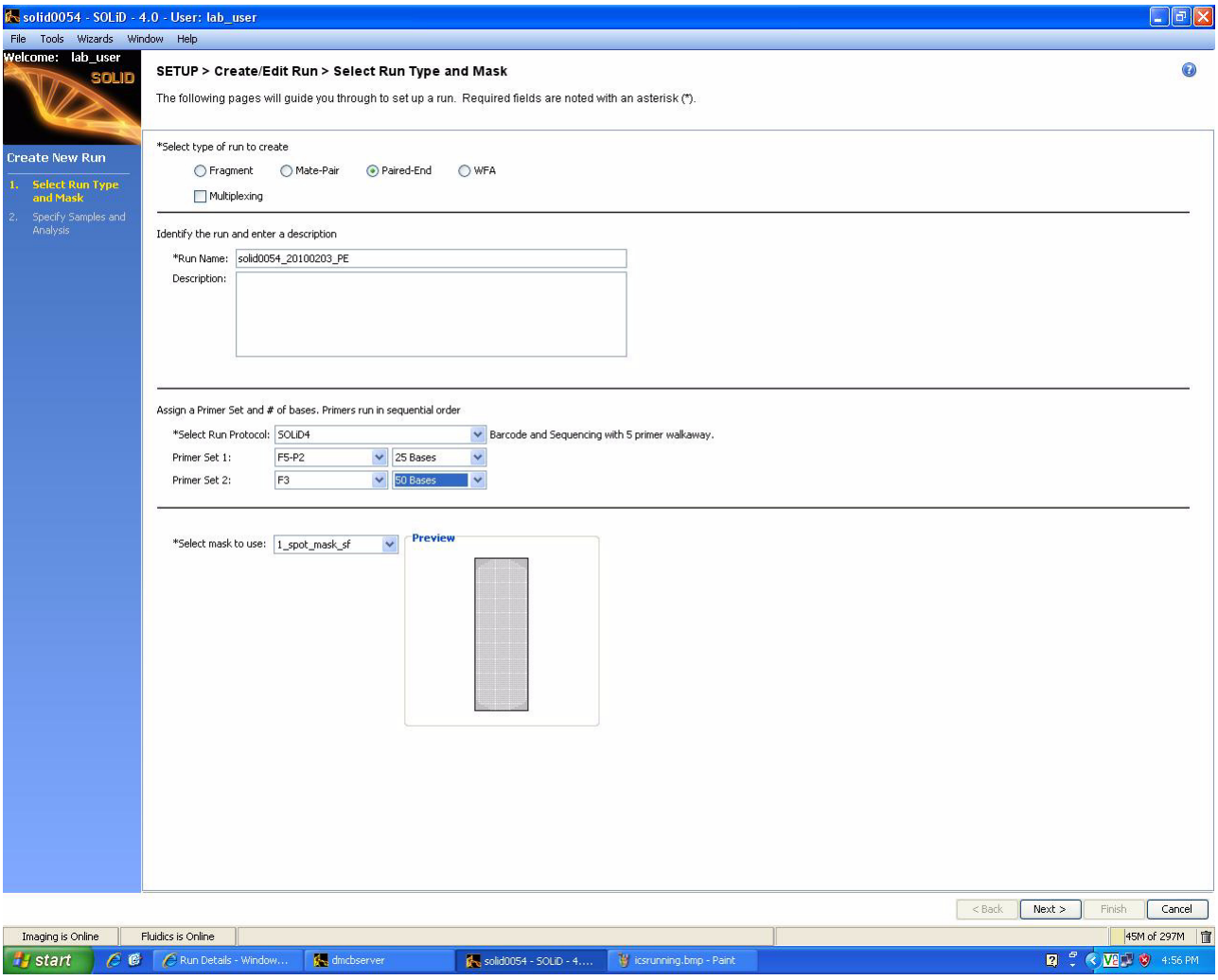
f. Enter read lengths for Primer Set 1, and if used, 2. A typical read length for the F3 and R3 tags is 35–50 bases. A typical read length for the F5-P2 tag or F5-BC tag is 35 bases.

g. Select the appropriate mask to use.

h. Click **Next**.

Note: If a message window appears during these steps, then continue setting up run as indicated. If a message remains after all selections have been made, correct the entries before clicking **Next**.

Figure 39 Complete the information in the Select Run Type and Mask pane.

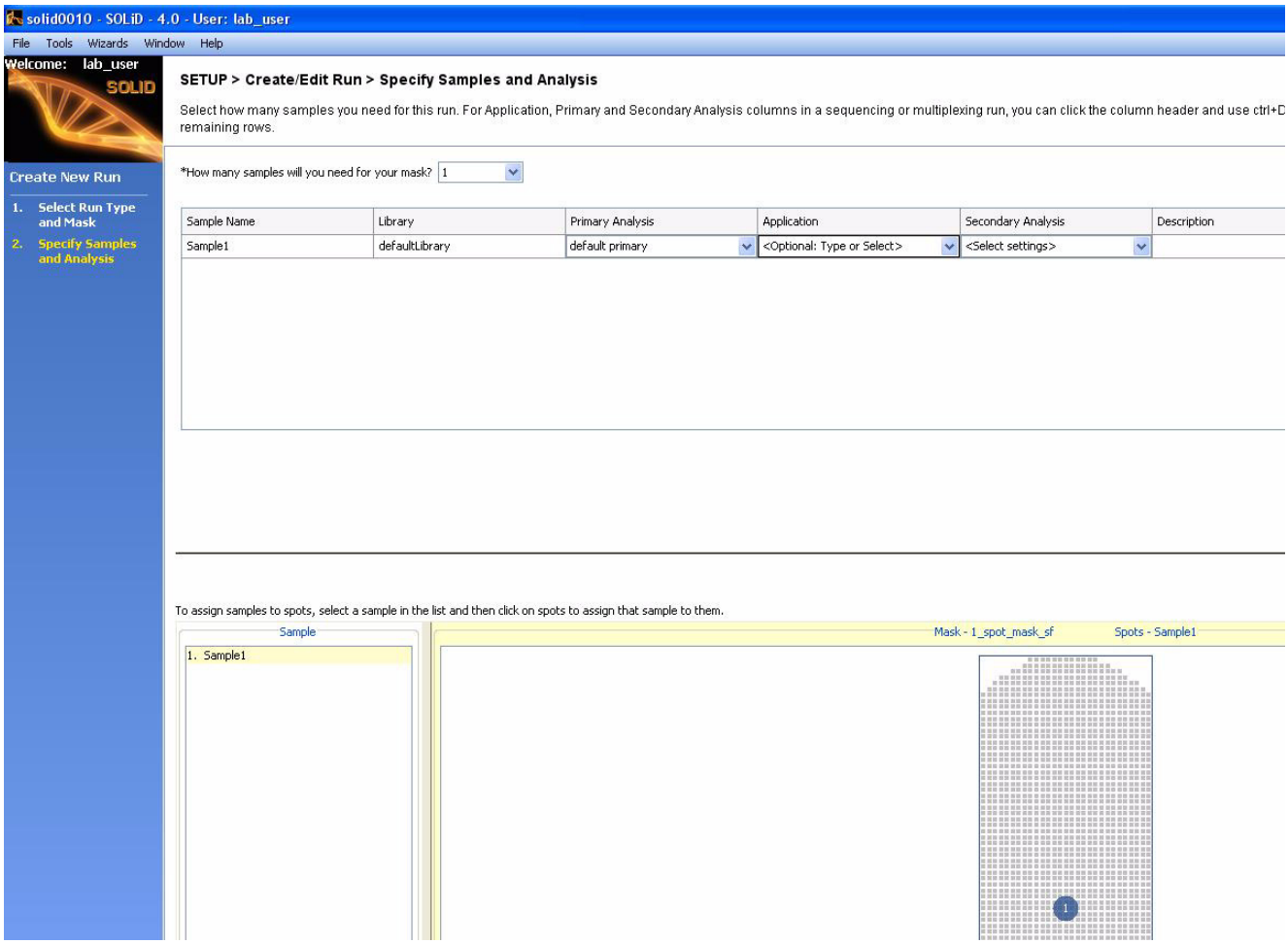

solid0054 - SOLiD - 4.0 - User: lab_user
File Tools Wizards Window Help
Welcome: lab_user
SOLID
SETUP > Create/Edit Run > Select Run Type and Mask
The following pages will guide you through to set up a run. Required fields are noted with an asterisk (*).
Create New Run
1. Select Run Type and Mask
2. Specify Samples and Analysis
*Select type of run to create
Fragment
Mate-Pair
Paired-End
WFA
Multiplexing
Identify the run and enter a description
*Run Name: solid0054_20100203_PE
Description:
Assign a Primer Set and # of bases. Primers run in sequential order
*Select Run Protocol: SOLiD4
Barcode and Sequencing with 5 primer walkaway.
Primer Set 1: F5-P2 25 Bases
Primer Set 2: F3 50 Bases
*Select mask to use: 1_spot_mask_sf
Preview
< Back
Next >
Finish
Cancel
Imaging is Online
Fluidics is Online
45M of 297M
start
Run Details - Window...
dmcbserver
solid0054 - SOLiD - 4....
icsrunning.bmp - Paint
4:56 PM

3. Complete the information in the Specify Samples and Analysis pane (see Figure 40):

a. Select the number of samples that will be used in the run. If a single sample will be run in multiple spots of the same slide, it counts as only one sample. Otherwise, the number of samples typically matches the number of spots on the mask.

b. (Optional) Edit the Sample Name, Library, Analysis settings, and Description.

Figure 40 Complete the information in the Specify Samples and Analysis pane.


solid0010 - SOLiD - 4.0 - User: lab_user
File Tools Wizards Window Help
Welcome: lab_user
SOLID
Create New Run
1. Select Run Type and Mask
2. Specify Samples and Analysis
SETUP > Create/Edit Run > Specify Samples and Analysis
Select how many samples you need for this run. For Application, Primary and Secondary Analysis columns in a sequencing or multiplexing run, you can click the column header and use ctrl+D remaining rows.
*How many samples will you need for your mask? 1
Sample Name
Library
Primary Analysis
Application
Secondary Analysis
Description
Sample1
defaultLibrary
default primary
<Optional: Type or Select>
<Select settings>
To assign samples to spots, select a sample in the list and then click on spots to assign that sample to them.
Sample
1. Sample1
Mask - 1_spot_mask_sf
Spots - Sample1
1

4. Assign Samples to spots in the mask on the Specify Samples and Analysis pane. A blue or white circle on the mask indicates that the numbered sample has been assigned to a spot. Clicking on a white circle selects that spot, and clicking on a blue circle un-assigns that spot. To assign a sample, select it from the Sample list, then click on a spot with no sample assigned to it.

5. Click **Finish** to return to the Setup Task pane.

6. Choose either to assign run to a flowcell for immediate use or to an instrument database to store for later use, then click **OK**.

7. To assign a run previously saved to the database:

   a. Click on **Manage Runs** in the task pane.

   b. Click the run, then select **Assign to Flowcell**.

c. Choose a flowcell, then click **OK**.

Create a multiplex sequencing run record

Note: For instructions to set up a sequencing (non-multiplex) run record, go to the previous section "Create a sequencing (non-multiplex) run record" on page 58.

There are two ways to create a multiplex sequencing run. The first method is to use the Run wizard (see below). The second method is to import a Run Definition file created offline (see "Set up a run by importing a Run Definition file" on page 105).

IMPORTANT! For all barcodes ≥ Barcode 16, ten bases of barcode tag must be read. While creating a multiplex sequencing run file on the System, the barcode definition file for the 1–96 series already specifies ten bases of sequence. If at least 1 library on the slide uses barcode 17 or higher, ten bases of barcode tag will be read for all other libraries on the slide, even if they are on separate spots.

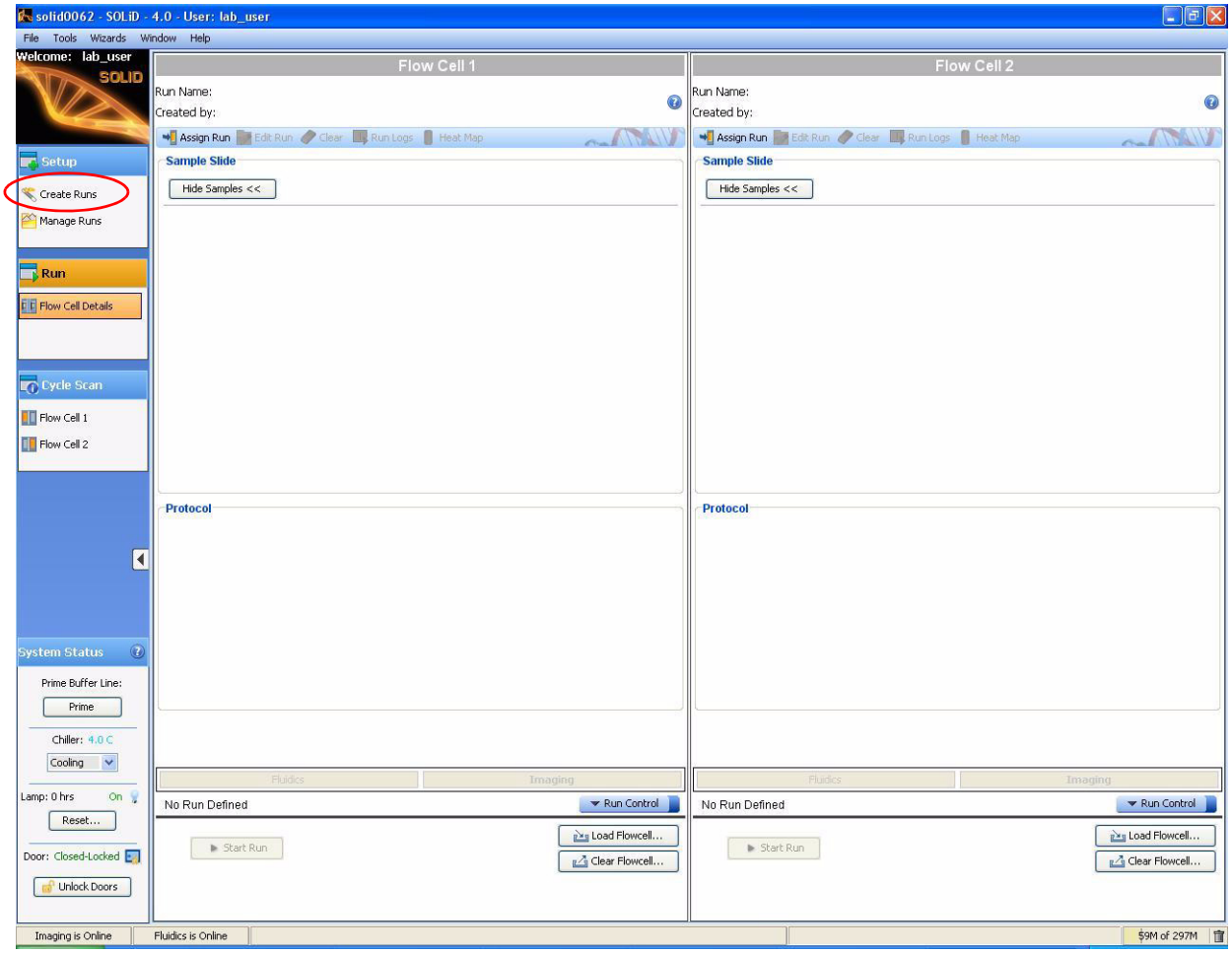
---

1. (Optional) If desired, modify the Barcode Error Correction Level. A Barcode Error Correction Level is set at 0 mismatches by default. A Barcode Error Correction Level of 1 mismatch increases the number of reads by about 10%, but lower-quality sequencing reads are included in the data.

   For information about how to modify the Barcode Error Correction Level, see "Modify the Barcode Error Correction Level" on page 120.

2. Click **Create Runs** in the Setup task pane on the left menu pane of the ICS (see Figure 41).

Figure 41 Use the run wizard to create a multiplex sequencing run.



3. Complete the information in the Select Run Type and Mask pane (see Figure 42):
   a. Select the type of run:
      - If sequencing only one end of the barcoded fragment library, select **Fragment**.
      - If sequencing both ends of the barcoded fragment library, select **Paired-End**.
   b. Ensure that the **Multiplexing** option is checked.
   c. (Optional) Type a new run name.
   d. (Optional) Enter a description.
   e. Ensure that the Run Protocol is set to **SOLiD4 Multiplex**.
   f. Select **BC** for Primer Set 1. Enter the read length for the BC Primer according to the barcodes used in the libraries:

Barcodes used on the slide	Set the read length to...(bases)
Barcodes 1–16 only	5
Barcodes 17–96	10

Note: If you do not select the correct read length for the number of barcodes specified in your run definition file, the SOLiD® System software will display an error. If at least 1 library on the slide uses Barcode 17 or higher, ten bases of barcode tag should be read.

g. Select Primer Set 2 (and Primer Set 3):
   - If sequencing only one end of the barcoded fragment library, select **F3** for Primer Set 2.
   - If sequencing both ends of the barcoded fragment library, select **F5-BC** for Primer Set 2 and **F3** for Primer Set 3.

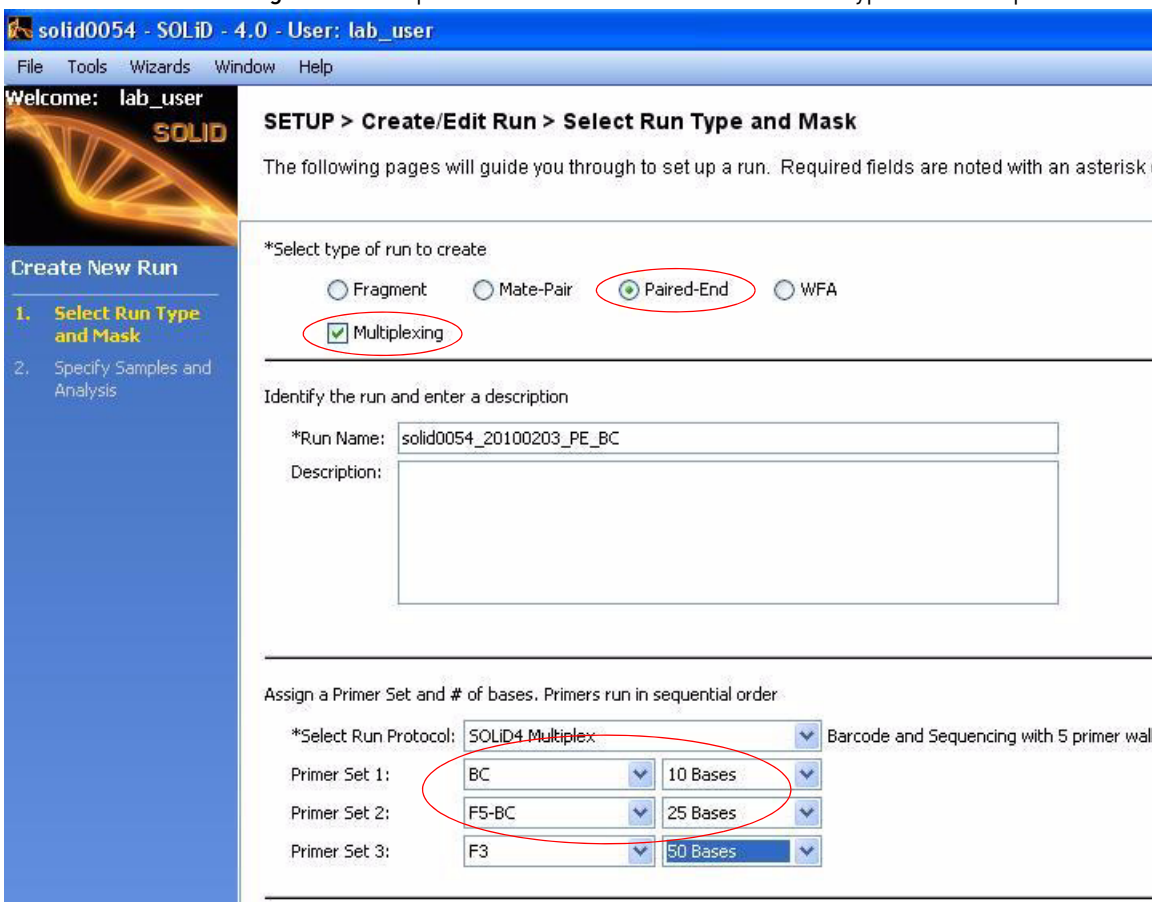
h. Enter read lengths for Primer Set 2, and if used, Primer Set 3. A typical read length for the F3 tag is 35–50 bases. A typical read length for the F5-BC tag is 25 bases.

   Note: For barcoded fragment libraries of short inserts such as small RNA samples, consult your Field Applications Specialist for the recommended sequencing reagents and read length.

i. Select the appropriate mask to use.

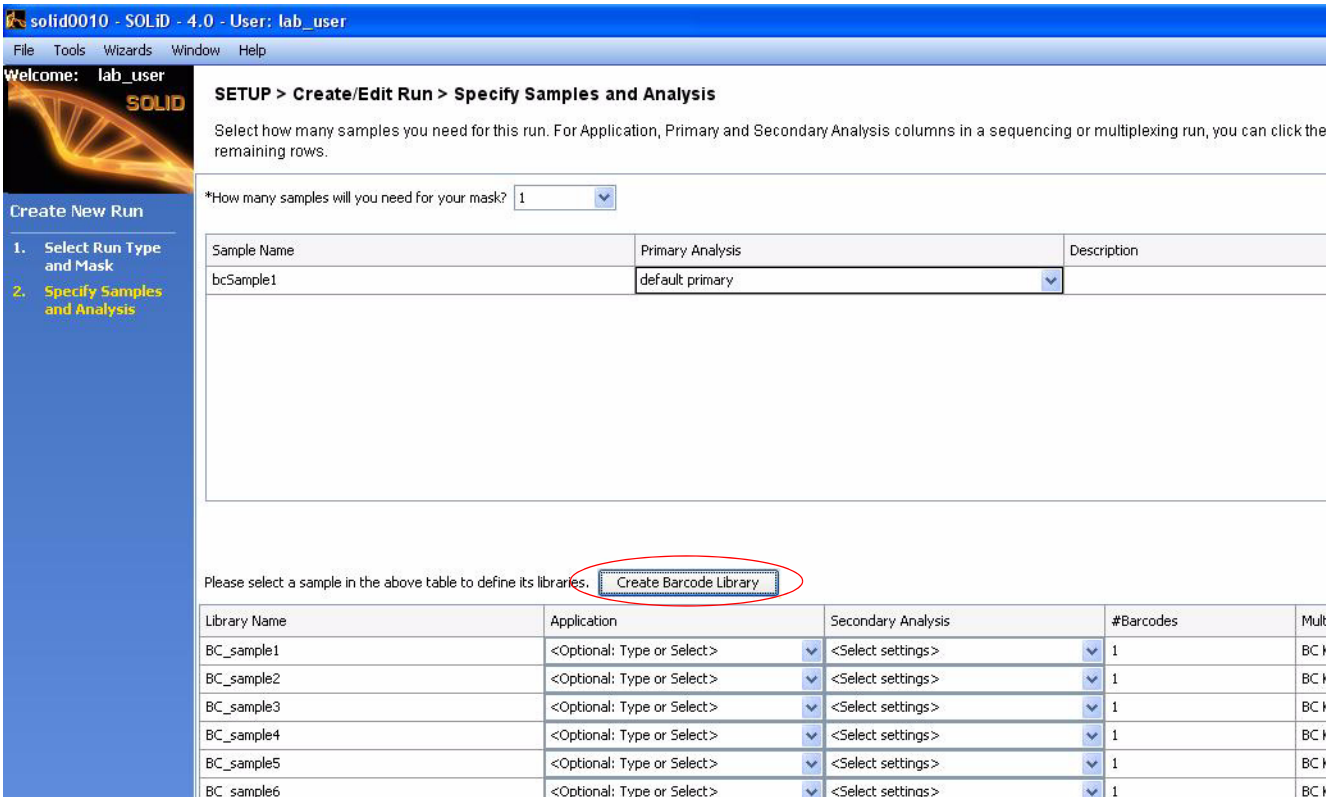
j. Click **Next**.

Figure 42 Complete the information in the Select Run Type and Mask pane.


solid0054 - SOLiD - 4.0 - User: lab_user
File Tools Wizards Window Help
Welcome: lab_user
SOLID
Create New Run
1. Select Run Type and Mask
2. Specify Samples and Analysis
SETUP > Create/Edit Run > Select Run Type and Mask
The following pages will guide you through to set up a run. Required fields are noted with an asterisk
*Select type of run to create
Fragment
Mate-Pair
Paired-End
WFA
Multiplexing
Identify the run and enter a description
*Run Name: solid0054_20100203_PE_BC
Description:
Assign a Primer Set and # of bases. Primers run in sequential order
*Select Run Protocol: SOLiD4 Multiplex
Barcode and Sequencing with 5 primer wal
Primer Set 1: BC 10 Bases
Primer Set 2: F5-BC 25 Bases
Primer Set 3: F3 50 Bases

4. Complete the information in the Specify Samples and Analysis pane (see Figure 43 on page 65):
   a. Select the number of samples that will be used in the run. A sample consists of a bead sample that may contain many barcoded libraries. A single sample that will run in multiple spots on the same slide counts as only one sample; otherwise, the number of samples typically matches the number of spots on the mask.
   b. Click the first sample name to select it. Change the sample name if desired. After you select the sample, you are able to enter the names, primary analysis, and description of libraries that are in the sample.

Figure 43 Complete the information in the Specify Samples and Analysis pane.


solid0010 - SOLiD - 4.0 - User: lab_user
File Tools Wizards Window Help
Welcome: lab_user
SOLID
Create New Run
1. Select Run Type and Mask
2. Specify Samples and Analysis
SETUP > Create/Edit Run > Specify Samples and Analysis
Select how many samples you need for this run. For Application, Primary and Secondary Analysis columns in a sequencing or multiplexing run, you can click the remaining rows.
*How many samples will you need for your mask? 1
Sample Name
Primary Analysis
Description
bcSample1
default primary
Please select a sample in the above table to define its libraries.
Create Barcode Library
Library Name
Application
Secondary Analysis
#Barcodes
BC_sample1
BC_sample2
BC_sample3
BC_sample4
BC_sample5
BC_sample6
<Optional: Type or Select>
<Select settings>
1

5. If performing a paired-end sequencing run, click **Create New Library** to begin entering library information for that sample (see Figure 43).

   For a barcode sample in a fragment sequencing run, select **Barcoded Sample** and click **Create New Library** to begin entering library information for that sample (see Figure 43).

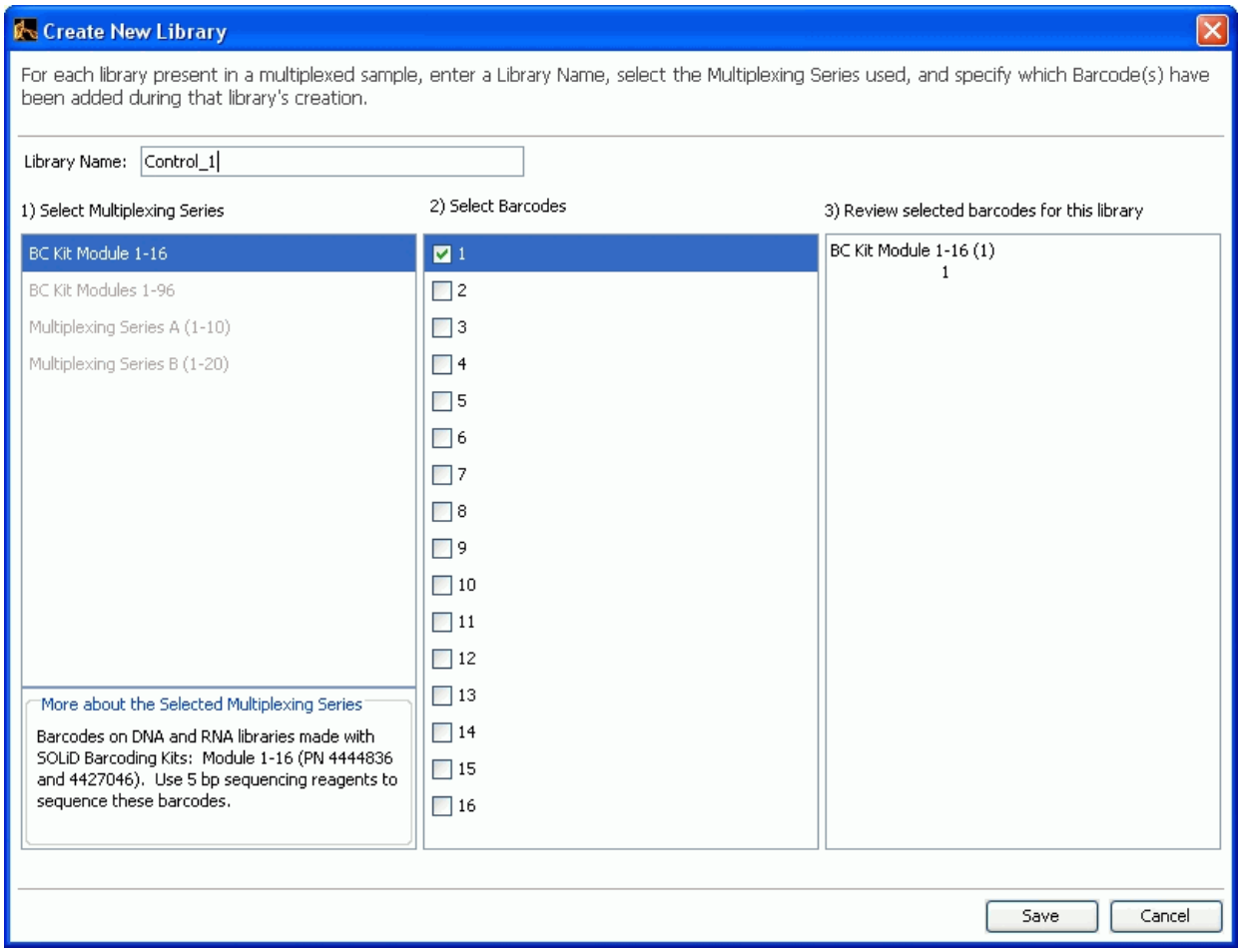
   IMPORTANT! Each barcoded sample requires at least one library.

   If there are any non-barcoded fragment library samples to be sequenced on the same slide as barcoded samples, select Non-barcoded Sample for that sample.

   Note: Non-barcoded samples cannot be sequenced on the same slide as barcoded samples for paired-end sequencing runs.

6. Enter information for the first library present in the sample (see Figure 44):
   a. Enter the library name.
   b. Select a Multiplexing Series.

Figure 44 Enter the information for the libraries in the sample.



IMPORTANT! Be sure to select the correct Multiplexing Series for your run. You can obtain additional information by selecting the Multiplexing Series and looking at the lower left panel: **More about the Selected Multiplexing Series**. If at least 1 library uses Barcode 17 or higher on the slide, you should select the **BC Kit Modules 1–96** option for each library on the slide.

Table 8 Contents of the Multiplexing Series†

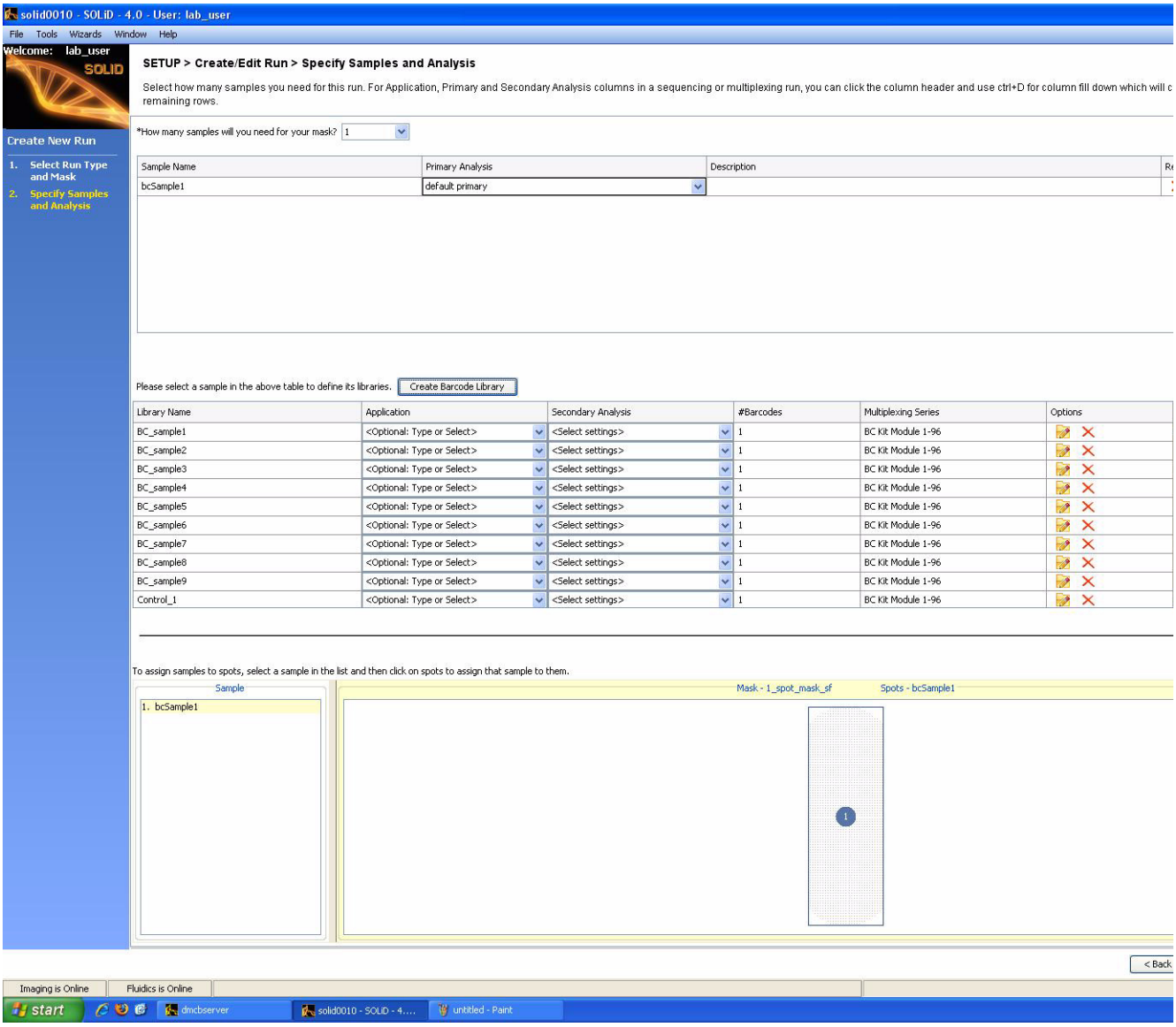
Multiplexing Series	Contents
BC Kit Module 1–16	16 barcodes in the SOLiD® Fragment Library Barcoding Kit Module 1–16 (Part no. 4444636) and SOLiD® Transcriptome Multiplexing Kit (Part no. 4427046)
BC Kit Modules 1–96	96 barcodes in the SOLiD® Fragment Library Barcoding Kit 1–96 (Part no. 4449637), or 48 barcodes in the SOLiD® RNA Barcode Modules
Multiplexing Series A (1–10)	10 barcodes that are in the SOLiD® Small RNA Expression Kit

† For the latest information regarding Multiplexing Series and barcode kits, contact your Field Applications Specialist.

Multiplexing Series	Contents
Multiplexing Series B (1–20)	20 barcodes that are supplied with the SOLiD® 3 System or 16 barcodes supplied with the SOLiD® 3 Plus System Use the "Multiplexing Series B (1–20)" option with the SOLiD® Transcriptome Multiplex Kit, which contains up to 16 barcodes

7. Click the selection box for the barcode or barcodes assigned to the library.
8. Click **Save** when you have completed the information for the first library.
9. (Optional) Repeat step 5 to step 7 for all remaining libraries. The Libraries panel of the Specify Samples and Analysis screen now shows information for all libraries for a particular sample (see Figure 45).

Figure 45 The Libraries panel shows information for all libraries.

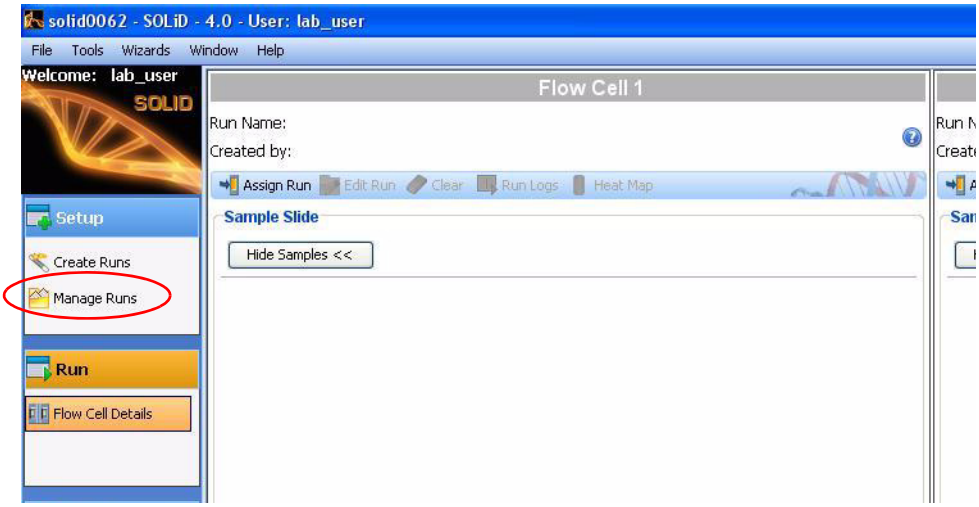


IMPORTANT! Review the Selected Barcodes for each library to ensure that they are correct, and be sure that the libraries are correctly assigned to the sample. After a run is started, you cannot change the barcode or library assignment.

10. Repeat step 5 to step 9 for all remaining samples.
11. Assign samples to unassigned spots in the mask. White circles are unassigned, and blue circles are assigned. To assign a sample:
    a. Select it from the Sample list.
    b. Click a white (unassigned) spot.

    To remove a sample from an assigned (blue) spot, click the spot. The sample is removed, and the spot turns white.
12. Once all of the samples have at least 1 library, click **Finish** and select **Save to Database**.
13. Click **Manage Runs** in the task pane on the left menu pane of the ICS (see Figure 46).

Figure 46 Manage Runs.



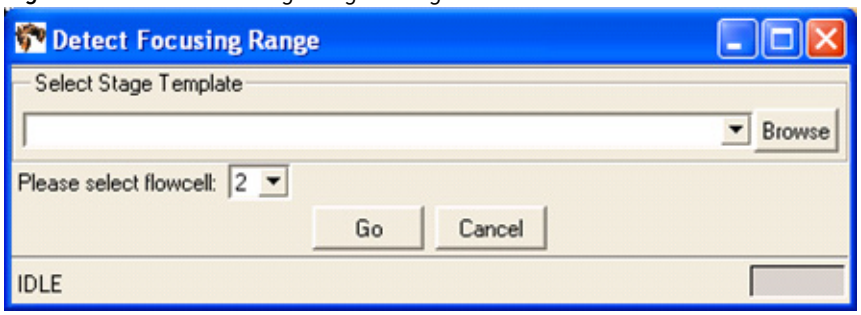
14. (Optional) To complete the information in the run record using a separate spreadsheet program, see "Set up a run by importing a Run Definition file" on page 105.
15. Choose to assign the run to a flowcell for immediate use or to an instrument database to store for later use, then click **OK**.
16. To assign a run previously saved to the database:
    a. Click on **Manage Runs** in the task pane.
    b. Click the run, then select **Assign to Flowcell**.
    c. Choose a flowcell, then click **OK**.

Detect the focus range

Note: If automatic focus range detection fails, determine the focus range manually (see "Manually find the focus range" on page 108).

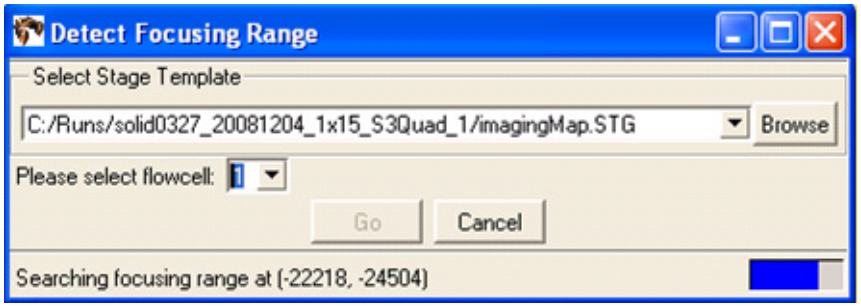
1. Close the front doors of the SOLiD® 4 Analyzer. Open the Imager window by choosing **Windows** ▸ **Imaging System**.
2. Select **Tools** ▸ **Detect Focusing Range**.
3. In the focusing range dialog, specify the stage template file by entering the name directly or by clicking the **Browse** button to navigate to a suitable one (see Figure 47). The stage template must match the slide in the target flowcell(s). If you created a run from the ICS, then select the file **imagingMap.STG** from the subdirectory in C:\Runs whose name matches the name of the run (for example, in Figure 48, C:\Runs\Solid0327_20081209_2_Oct_Test\imagingMap.STG is selected).

Figure 47 Detect Focusing Range dialog.



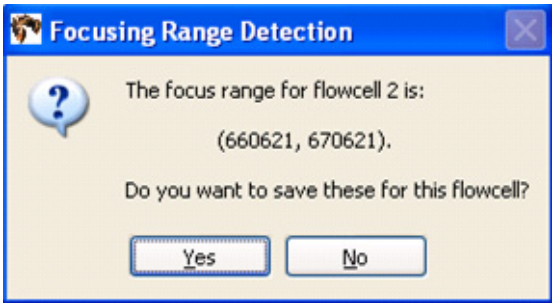
4. Select the flowcell using the drop-down menu, then press **Go**. The Imager works for several minutes while it determines the range. The blue progress bar indicates how close it is to completion (see Figure 48). You can also click **Cancel** so that the Imager aborts the ranging operation.

Figure 48 Detect Focusing Range Dialog while detection is in progress.



5. When the Imager is done, a dialog appears (see Figure 49). Click **Yes** if you want to replace the values in the local settings file. Click **No** if you want the Imager to discard the newly calculated focus range.

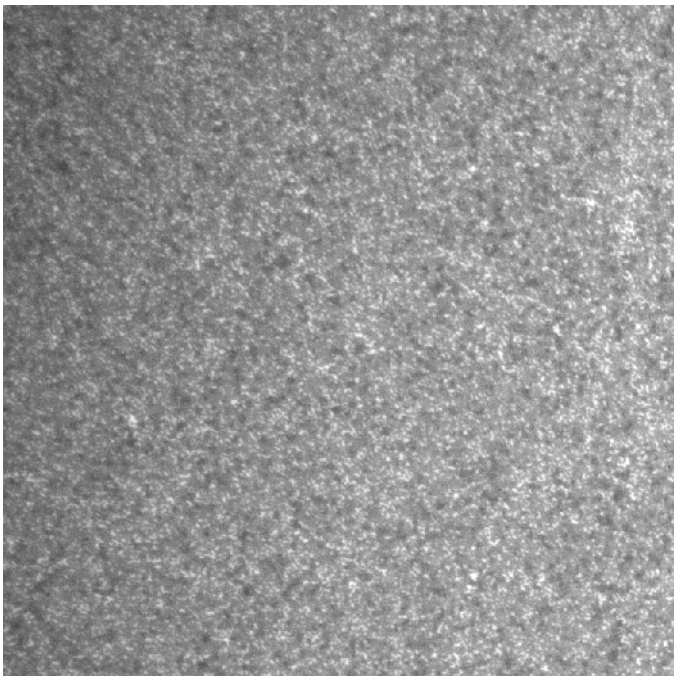
Figure 49 Confirm that you want to replace the local settings file.



6. Verify the validity of the newly calculated focus range by taking images at random locations.

IMPORTANT! You should see images of beads, ensuring that the algorithm was able to focus on the beads and not on other artifacts (see Figure 50). If you do not see bead images or if you see out-of-focus bead images, set the focus range manually (see "Manually find the focus range" on page 108).

Figure 50 Beads in focus.



Start the sequencing run

Note: Before starting the run, make sure that the air filter at the back of the instrument is not clogged with dust (see "Clean the air filter" on page 98).

1. Ensure that there is adequate disk space for images and results of the sequence run (for the minimum needed disk space, see Table 9). To know the amount of disk space that is available, click **Manage Runs** on the task pane.

Table 9 Minimum required disk space to store images and results

Run Type	Minimum space needed for images	Minimum space needed for Primary Analysis results (spch, csfasta, QV.qual)	Minimum space needed for Secondary Analysis results (mapping/pairing)
Fragment (35 bp)	2.7 TB	1.3 TB	<200 GB
Fragment (50 bp)	3.8 TB	1.8 TB	<200 GB
Mate-paired (25/25 bp)	3.8 TB	1.8 TB	<200 GB
Mate-paired (35/35 bp)	5.3 TB	2.4 TB	<200 GB
Mate-paired (50/50 bp)	7.6 TB	3.3 TB	<200 GB
Paired-end (50/35 bp)	6.5 TB	3.1 TB	<200 GB
Multiplex fragment (35/5 bp)	3.1 TB	1.5 TB	<200 GB
Multiplex fragment (35/10 bp)	3.4 TB	1.7 TB	<200 GB
Multiplex fragment (50/5 bp)	4.2 TB	2.1 TB	<200 GB
Multiplex fragment (50/10 bp)	4.6 TB	2.3 TB	<200 GB
Multiplex paired-end (50/35/5 bp)	6.9 TB	3.3 TB	<200 GB
Multiplex paired-end (50/35/10 bp)	7.3 TB	3.5 TB	<200 GB

2. Click **Start Run**.
3. If there is not enough room to store the data for the run, then the Start Run dialog box appears (see Figure 51). Choose the appropriate option (see Table 51).

Figure 51 This Start Run dialog appears if there is not enough room to store data for the run.

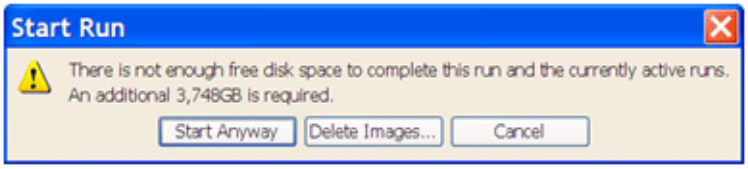

Start Run
There is not enough free disk space to complete this run and the currently active runs.
An additional 3,748GB is required.
Start Anyway
Delete Images...
Cancel

Table 10 Choose one of the three options to manage disk space

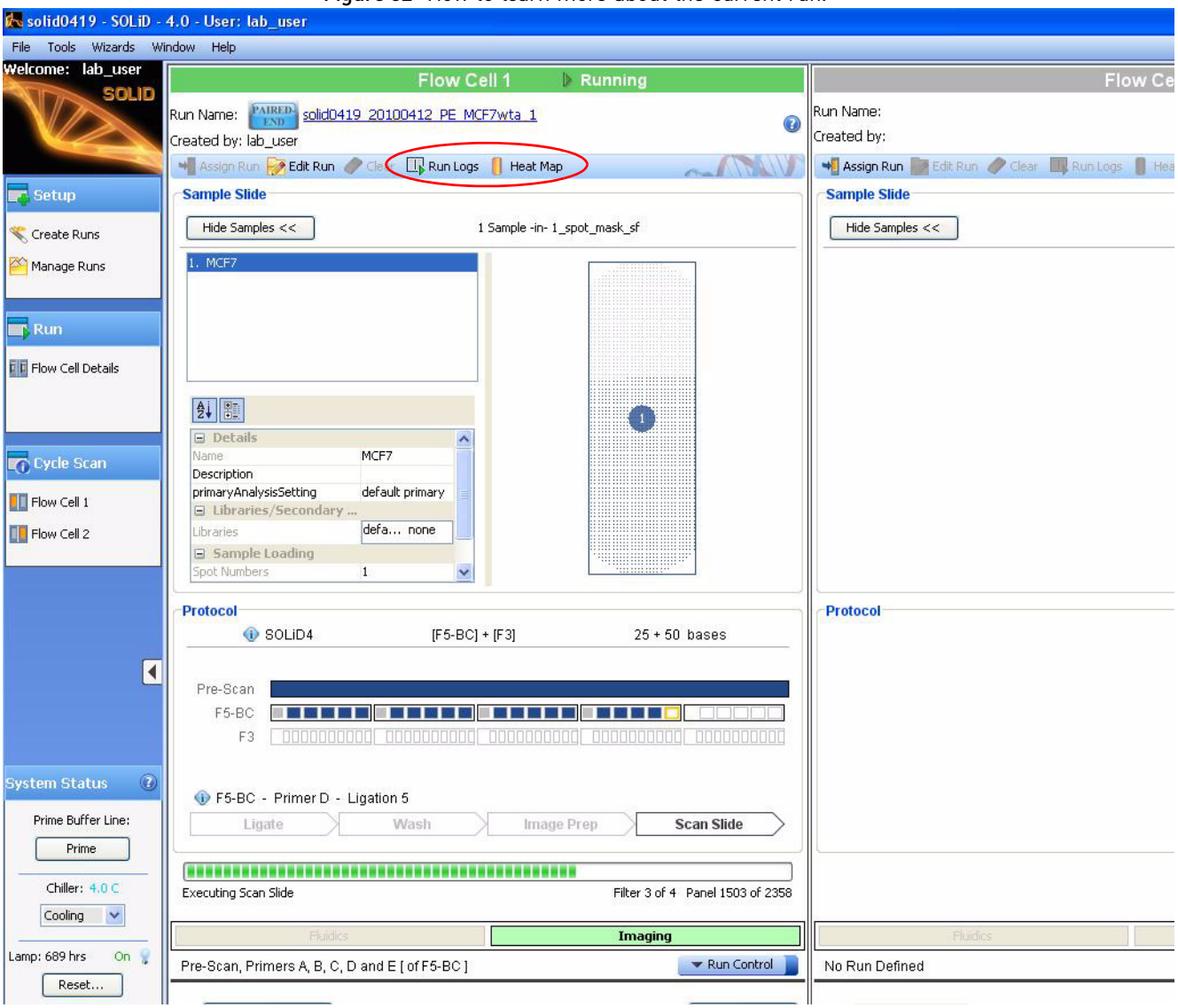
Option	Description
Start Anyway	Initiates the run. The instrument pauses itself when it runs out of free disk space.
Delete Images†	Launches Historical Runs page in SETS. Images and/or results can be deleted through SETS
Cancel	Aborts the run.

† For more information on creating more available disk space, see the *Applied Biosystems SOLiD® 4 System SETS Software User Guide* (Part no. 4448411).

IMPORTANT! Before deleting an image, ensure that data analysis from the previous run is satisfactory and complete. For more information, refer to the *Applied Biosystems SOLiD® 4 System SETS Software User Guide* (Part no. 4448411).

4. After the run has been initiated, you can click the **Run Log** and **Heat Map** buttons located at the top of the appropriate flowcell panel to learn more information about the current run (see Figure 52). You can also select the appropriate flowcell in the Cycle Scans menu on the task bar on the left.

Figure 52 How to learn more about the current run.



IMPORTANT! Do not disturb the SOLiD® 4 Analyzer while in operation and do not open the flowcell during a pause in the run. Significant perturbations (for example, opening system parts, vibrations) during the run are detrimental to the results.

Control the run

Pause/Resume Run

Note: Special considerations need to be made when using the Pause Run/Resume Run function in certain modules:

When the run has been paused in the middle of or at the end of the Scan Slide module, and if the Scan Slide module of the same cycle needs to be repeated, it is recommended that you resume the run starting from the Image Prep module.

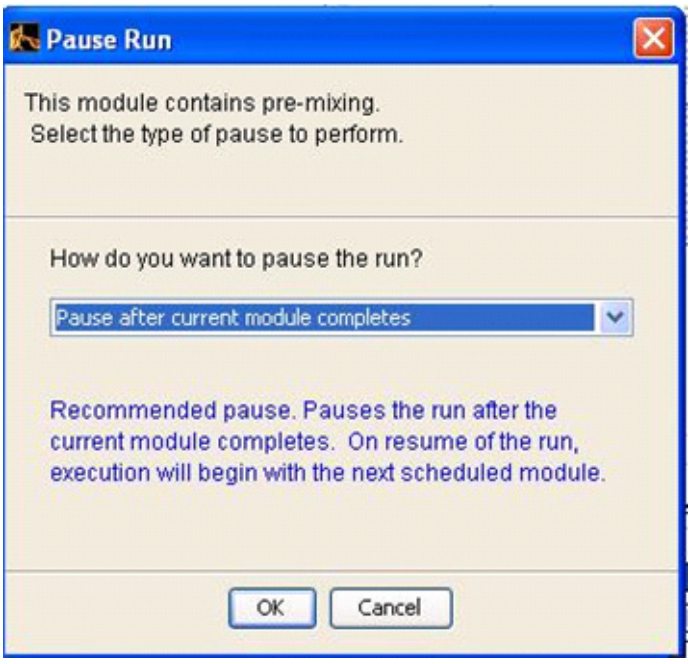
Pausing the run is generally followed by automatic filling of the flowcell with Storage Buffer. Resuming from the Image Prep module fills the flowcell with Imaging Buffer again, which is required to perform the functions in the Scan Slide module. You can resume from the Image Prep module using the Change Run Progress Point command in the Run Control menu (see "Use the Run Control menu" on page 74).

Eleven modules contain a pre-mixing step for the next module:

- Prime/Ligate Mix
- Ligate/Dark Ligate Mix
- Dark Ligate/Phosphatase Mix
- Cleave/Ligate Mix
- Prime/Bridge Ligate Mix
- Bridge Ligate/Phosphatase Mix
- Dark Ligate/Capper Enzyme Mix
- Cleave/RevPhosphatase Mix
- RevPhosphatase/Ligate Mix
- RevPhosphatase/Bridge Ligate Mix
- Bridge Ligate/Capper Enzyme Mix

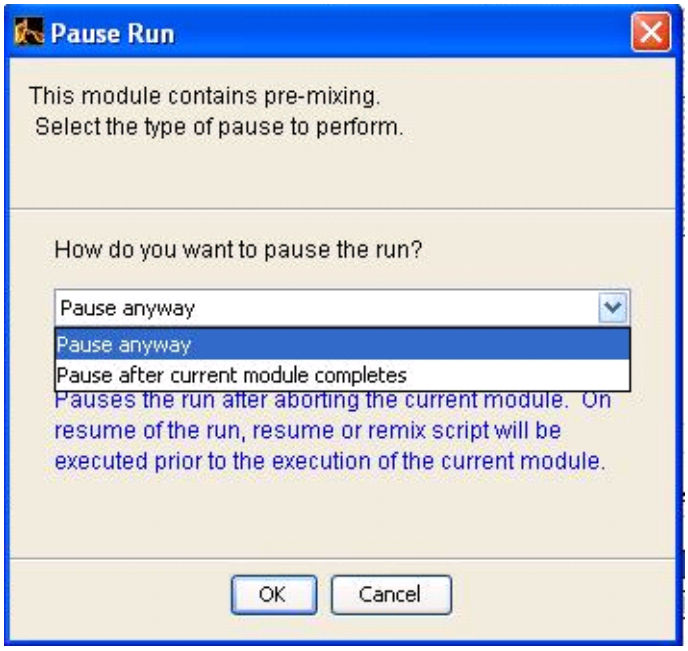
When you try to pause a run in the middle of the module, a dialog displays (see Figure 53).

Figure 53 Pause Run dialog for pre-mixing module.


Pause Run
This module contains pre-mixing.
Select the type of pause to perform.
How do you want to pause the run?
Pause after current module completes
Recommended pause. Pauses the run after the current module completes. On resume of the run, execution will begin with the next scheduled module.
OK
Cancel

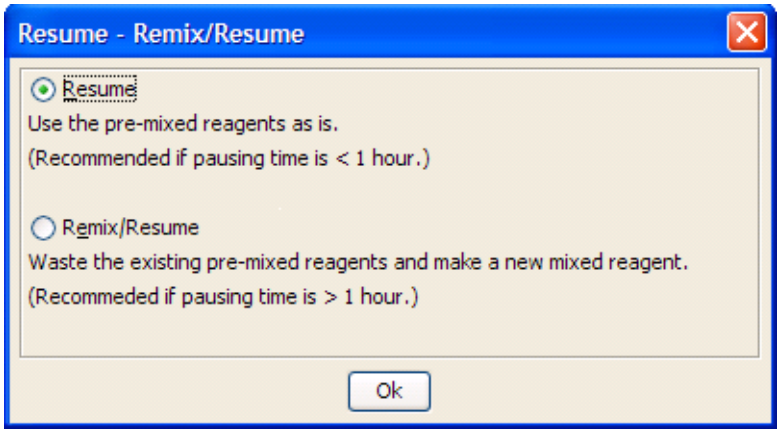
The default setting is to pause after the current module completes so that pre-mixing is not interrupted. If you want to pause the run within a pre-mixing module, select **Pause anyway** (see Figure 54).

Figure 54 Pause Run dialog options for pre-mixing module.


Pause Run
This module contains pre-mixing.
Select the type of pause to perform.
How do you want to pause the run?
Pause anyway
Pause anyway
Pause after current module completes
Pauses the run after aborting the current module. On resume of the run, resume or remix script will be executed prior to the execution of the current module.
OK
Cancel

When you resume a run to use the pre-mixed reagents in the strip tube, a dialog box displays (see Figure 55). While it is recommended to simply resume the run, the system provides an option to remix the reagents by clicking **Remix/Resume**. In this case, the previous pre-mixed reagent is delivered to the waste and a new reagent is mixed.

Figure 55 The options to resume or remix/resume the run are available.

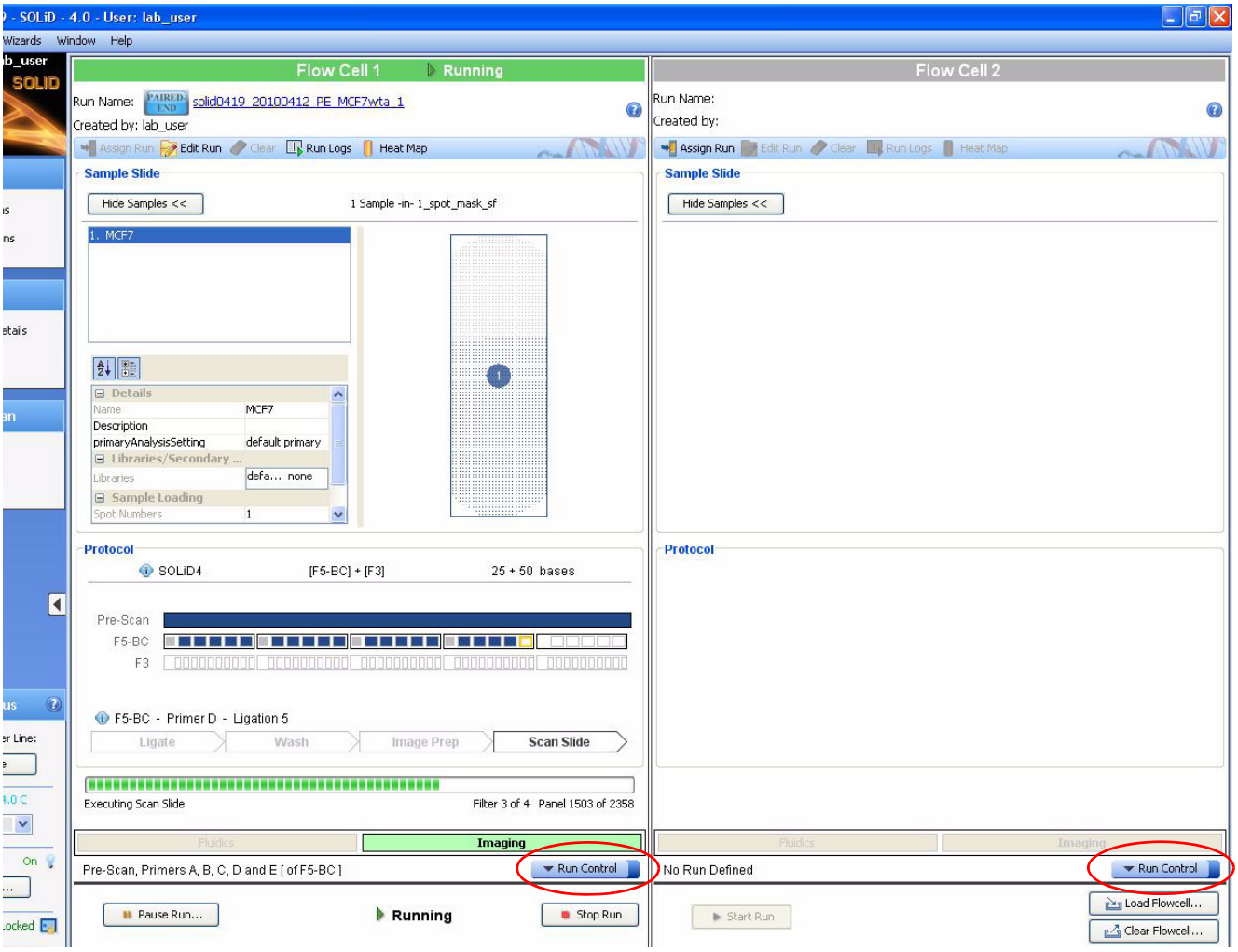


Note: One reagent strip contains reagents enough for 10 cycles per primer. Therefore, if the remix option is used, the cycle number which can be used with the reagent strip is reduced.

Use the Run Control menu

1. Click **Run Control** to display the Run Control menu (see Figure 56).

Figure 56 Click **Run Control** to display the Run Control menu for that flowcell.



Run Control commands

IMPORTANT! Do not disturb the SOLiD® 4 Analyzer while in operation and do not open the flowcell during a pause in the run. Significant perturbations (for example, opening system parts, vibrations) during the run are detrimental to the results.

Table 11 Run Control commands

Command†	Command is available while the instrument is...
Stop Run	Running or paused
Reset Current Primer	Paused within Primer Cycle
Change Primer Schedule	Paused
Set Early Pause Point	Running or paused
Change Run Progress Point	Paused

† Certain commands have sub-menus that allow you to control every step in a run protocol.

Stop Run

The Stop Run command launches a Stop Run dialog box. Choose the appropriate option in the dialog according to Table 12.

Table 12 Options available in the Stop Run dialog

Option	Description
Abort Run	Run aborts and analysis jobs are cancelled.
Set run as completed	Run is set as completed. Secondary analysis jobs start.

Change Primer Schedule

The Change Primer Schedule command allows you to choose a first and second primer, in any order. Depending on how you set up the run, only primers from a selected primer set are available in the sub-menu.

Set Early Pause Point

The Set Early Pause Point command allows you to define when the instrument pauses. This command can be used to replenish reagents at a more convenient time than the time defined by the software. Note that if the Change Run Progress Point command (see below) is selected, any changes to the Early Pause Point resets to the default.

Change Run Progress Point

The Change Run Progress Point command allows you to back up or skip to any specific point in the run. Ensure that you select points that are consistent with the progress of the run.

Repeating primers can be performed by using the Change Run Progress Point command, selecting a primer to repeat, resuming the run, and using the Set Early Pause Point command to pause the run after the repeated primer has completed.

Use imaging and analysis controls for specific spots

The Instrument Control Software (ICS) allows you to control imaging for individual spots on a slide. With the controls, you can deposit fragment and mate-paired library samples onto the same slide and turn off imaging of a spot. To access the imaging and analysis controls, in the Sample Slide display of the ICS, right-click a sample name to display options menu. These options are not available until the focal map (prescan) is complete.

The options menu includes:

- Enabled
- Imaging Turned Off
- Spot discarded

Enabled

Enabled is the default setting and allows for both imaging and analysis of the spot.

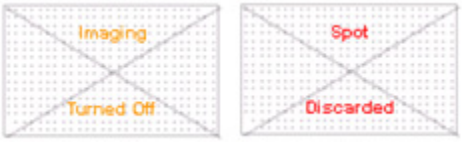
Imaging Turned Off

Imaging Turned Off turns off imaging but allows analysis to complete.

Spot Discarded

Spot Discarded turns off both imaging and analysis. Spot Discarded can be used to remove problematic samples from the software workflow. Use of Spot Discarded updates the Sample Slide display according to the selection in Spot Discarded. Use of Spot Discarded also affects subsequent cycles (see Figure 57).

Figure 57 The Sample Slide display updates after using Imaging Turned Off or Spot Discarded.



Monitor the run

Note: To monitor the run remotely, use SETS from any computer to connect to the networked instrument. Refer to the *Applied Biosystems SOLiD® 4 System SETS Software User Guide* (Part no. 4448411). Set up optional email notification regarding instrument run and system information using SETS. Refer to the *Applied Biosystems SOLiD® 4 System SETS Software User Guide*.

View the run log

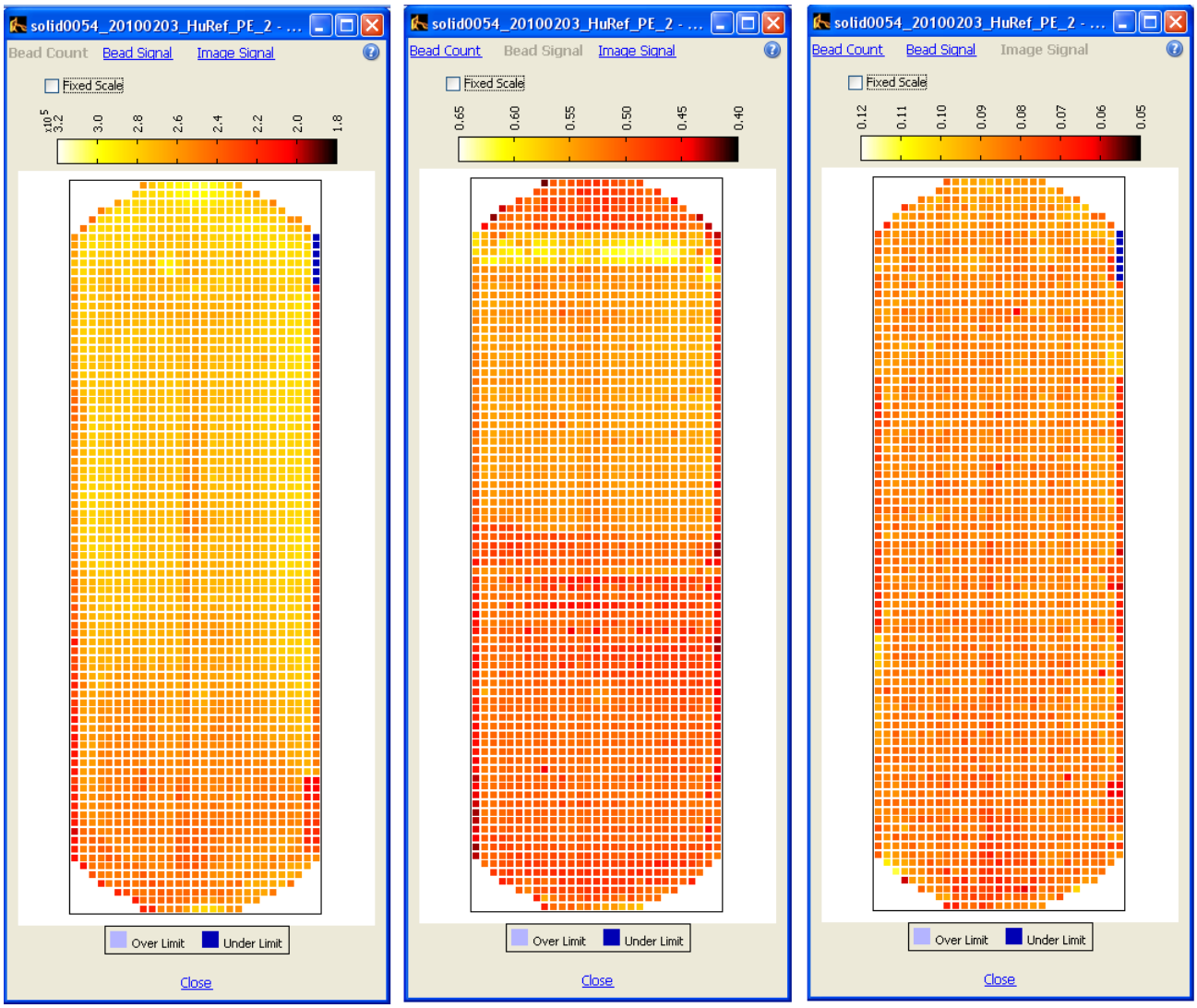
1. Click **Run Log** located at the top right corner of the flowcell panel to open a dialog box describing a series of instrument events.
2. After you finish viewing the Run Log, click **Close** located at the bottom of the Run Log window.

View the heat maps

1. To view the heat map showing bead densities found in the focal map images, click **Heat Map** located at the top right corner of the flowcell panel (see Figure 58).
2. Look for:
   - Uniform deposition of beads on the slide.
   - The actual average bead deposition density/panel value being similar in value to the targeted average bead deposition density (for example, 300,000 beads/panel). A large number of missing panels could indicate a deposition problem.

     Note: The heat map is not available until after the completion of the first sequencing cycle. The software refines the focal map using images collected during the first sequencing cycle and does not display the heat map until after all of the images collected during both the Prescan and cycle 1 have been processed. This may take up to 30 minutes depending on the number of panels that were imaged.
3. After viewing the Heat Map, click **Close** located at the bottom of the Heat Map window.

Figure 58 Bead Count (left), Bead Signal (center), and Image Signal (right) heat maps.



View cycle scans

1. Select the appropriate flowcell (**Flow Cell 1** or **Flow Cell 2**) in the Cycle Scan menu on the task bar on the left.

   The top section of the Cycle Scans window lists all the scans per ligation cycle for the slide, and the bottom section shows scan information sorted by sample for the scan selected in the top section. Use the parameters shown in Figure 59 to assess the progress of the sequencing run (see Table 13).

Figure 59 Parameters available in the Cycle Scans window to assess the progress of the sequencing run: 1 Failed panels; 2 Best + Good Beads / usable beads; 3a/3b Effective exposure times; 4 Satay plots.

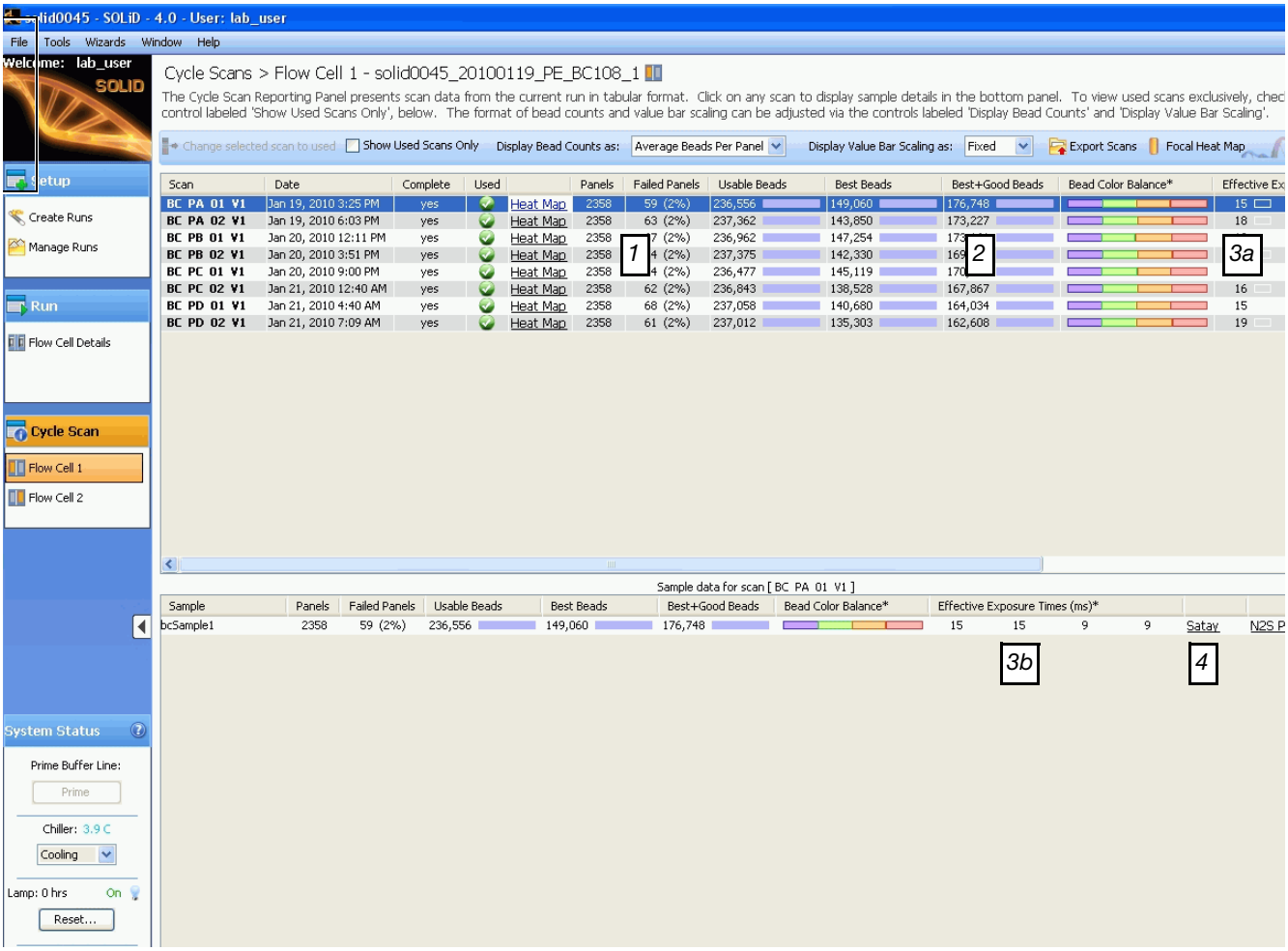

File Tools Wizards Window Help
Welcome: lab_user
Cycle Scans > Flow Cell 1 - solid0045_20100119_PE_BC108_1
Show Used Scans Only
Display Bead Counts as:
Average Beads Per Panel
Display Value Bar Scaling as:
Fixed
Export Scans
Focal Heat Map
Setup
Create Runs
Manage Runs
Run
Flow Cell Details
Cycle Scan
Flow Cell 1
Flow Cell 2
System Status
Prime Buffer Line:
Prime
Chiller: 3.9 C
Cooling
Lamp: 0 hrs
On
Reset...
Scan
Date
Complete
Used
Panels
Failed Panels
Usable Beads
Best Beads
Best+Good Beads
Bead Color Balance*
Effective Ex
BC PA 01 V1 Jan 19, 2010 3:25 PM yes Heat Map 2358 59 (2%) 236,556 149,060 176,748 15
BC PA 02 V1 Jan 19, 2010 6:03 PM yes Heat Map 2358 63 (2%) 237,362 143,850 173,227 18
BC PB 01 V1 Jan 20, 2010 12:11 PM yes Heat Map 2358 236,962 147,254
BC PB 02 V1 Jan 20, 2010 3:51 PM yes Heat Map 2358 237,375 142,330
BC PC 01 V1 Jan 20, 2010 9:00 PM yes Heat Map 2358 236,477 145,119
BC PC 02 V1 Jan 21, 2010 12:40 AM yes Heat Map 2358 62 (2%) 236,843 138,528 167,867 16
BC PD 01 V1 Jan 21, 2010 4:40 AM yes Heat Map 2358 68 (2%) 237,058 140,680 164,034 15
BC PD 02 V1 Jan 21, 2010 7:09 AM yes Heat Map 2358 61 (2%) 237,012 135,303 162,608 19
1
2
3a
Sample data for scan [BC PA 01 V1]
Sample
Panels
Failed Panels
Usable Beads
Best Beads
Best+Good Beads
Bead Color Balance*
Effective Exposure Times (ms)*
bcSample1 2358 59 (2%) 236,556 149,060 176,748 15 15 9 9 Satay N2S P
3b
4

Table 13 Cycle Scans window: Distinguish normal runs from problematic runs

Parameter	Normal run	Problematic run
(1) Failed panels (number of panels that failed image alignment during color-calling).	In general, the number of failed panels should be relatively small and consistent.	Run begins with extremely high number of failed panels or dramatic increase in any subsequent ligation cycle for each sequencing primer.
(2) The fraction of (Best + good beads)/usable beads	The fraction can vary depending on the quality of the library, the efficiency of the PCR, and the enrichment process. As a guideline, the fraction is around 0.5–0.6 in the first ligation cycle of each primer and drops to 0.2–0.3 in the last cycle.	A significant drop in the fraction of good beads in the initial ligation cycles would indicate a reason to pause the run and to troubleshoot the performance.

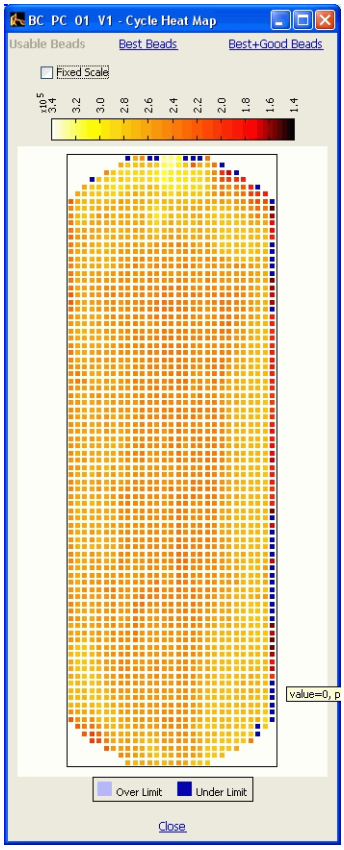
Parameter	Normal run	Problematic run
(3a/3b) Effective exposure times†	Gradual increase from ligation cycles 1–5 or higher for each sequence primer. Performance varies from slide to slide and as a function of the age of the SOLiD® Light Source.‡ As a guideline, the effective exposure time is typically 20–40 ms in the first ligation cycle and increases to 100–300 ms in the tenth cycle.	250 ms or greater in ligation cycle 1 or when instrument times out when the effective exposure time exceeds 500 ms. Long exposure times may indicate replacement of the SOLiD® Light Source‡.
(4) Satay plots	The first cycle of any primer should show a relatively "clean" Satay plot, with most points clustered on the four color axes and with minimal fraction of the points clustered around the origin. The quality of the Satay plot typically degrades gradually with each ligation cycle for a single primer cycle, becoming more "fuzzy" in the last cycle.	An abnormal "fuzzy" Satay plot in the first cycle is a reason to pause the run and troubleshoot the performance.

† Exposure time is indicative of the signal intensity of the beads. The instrument uses an auto-exposure routine, on a per sample basis, to maximize bead signal with minimal image saturation. Shorter exposure times are associated with efficient ligation of the fluorescent probes.

‡ You should replace the SOLiD® Light Source in the Life Technologies SOLiD® 4 Analyzer every 1500 hours of use. See Appendix B for instructions.

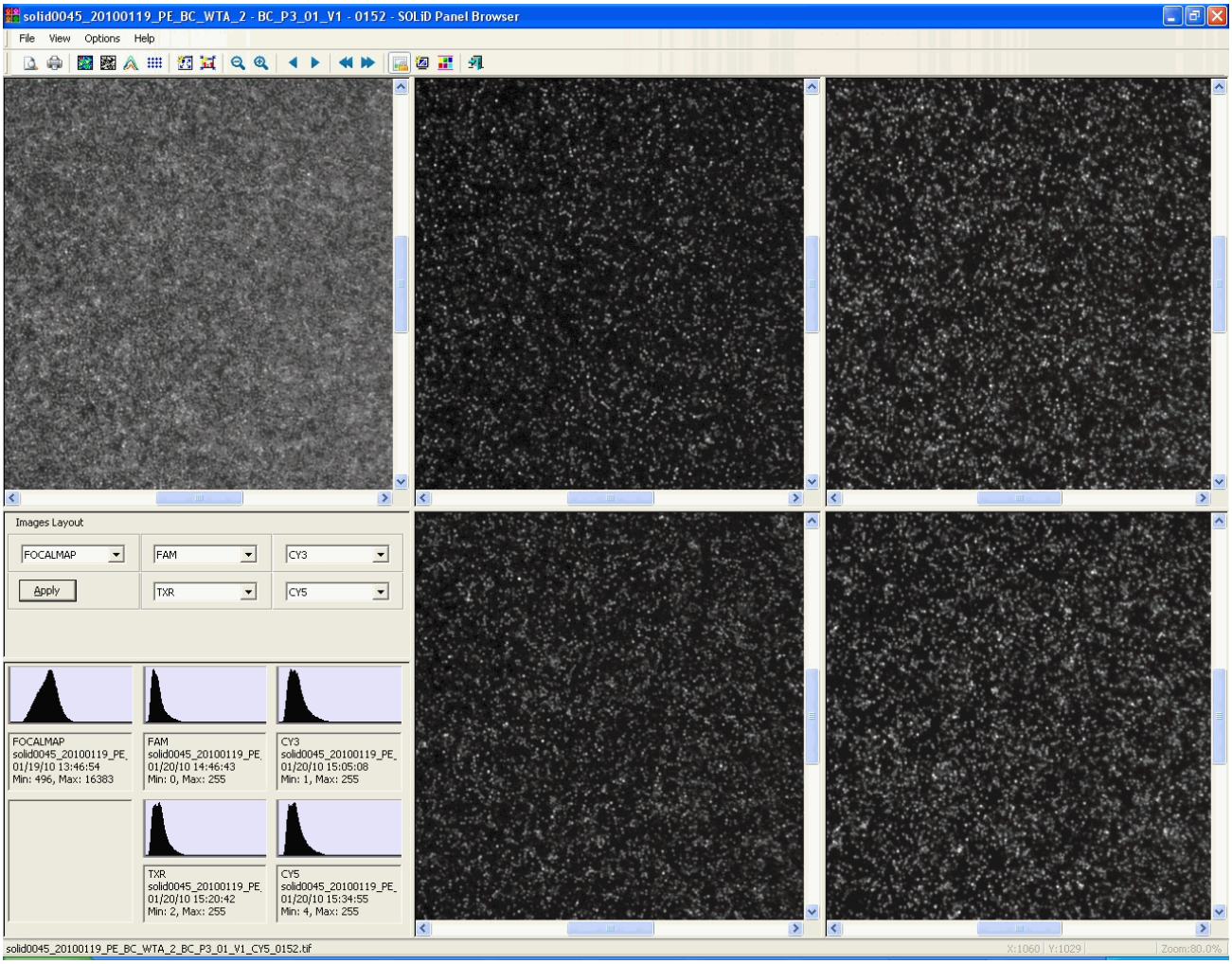
2. Click the heat map link to view the heat map for that cycle (see Figure 60).

Figure 60 Click the heat map link to view the heat map for that cycle.



3. Left-double-click any square panel on the heat map to open the panel browser window. The panel browser allows you to view the focal map and the image for each fluorescent dye signal (see Figure 61).

Figure 61 View the focal map and the image for each fluorescent dye signal.



4. After you finish viewing the Cycle Scans, close the Cycle Scans window.

   Note: If the sequencing run appears problematic, you can: (1) Allow the run to continue and troubleshoot after the run or (2) Pause the run and troubleshoot. Consult a SOLiD® System Field Applications Specialist.

A

Applied Biosystems SOLiD® 4 System Instrument Operation Guide Required Materials

This appendix covers:

- Set up and perform a workflow analysis (WFA) run 83
- Required Applied Biosystems reagent kits. 83
- Required equipment. 84
- Required consumables. 86
- Set up and perform a sequencing run 86
- Required Applied Biosystems reagent kits. 86
- Required equipment. 91
- Required consumables. 93

Set up and perform a workflow analysis (WFA) run

Required Applied Biosystems reagent kits

Table 14 Required Applied Biosystems reagent kits: WFA run

Item (part number)	Components	Kit component(s) used in...
SOLiD® ToP Instrument Buffer Kit (4452688)	Reset Buffer Glycerol 10X Instrument Buffer Cleave Solution 1 Cleave 2.1 Kit (Cleave 2.1 Parts 1 and 2) Storage Buffer Imaging Buffer Kit (Imaging Buffer Parts 1 and 2) 1X T4 Ligase Buffer Kit (1X Ligase Buffer Parts 1 and 2) Universal Buffer Kit (Universal Buffer Parts 1 and 2)	Sequencing and/or WFA
SOLiD® XD Slide & Deposition Kit v2 (4456997)	4 × 2 Sequencing Slides Slide Deposition Buffer v2 Slide Storage Buffer	Bead deposition
SOLiD® ToP Workflow Analysis Reagents (4453237)	SOLiD® ToP Workflow Analysis Reagents	WFA

Item (part number)	Components	Kit component(s) used in...
SOLiD® Flowcell O-rings (4398217)	10-pack of flowcell O-rings	Sequencing and/or WFA

Required equipment

Table 15 Required equipment: WFA run

Item†	Source
SOLiD® 4 System	Applied Biosystems PN 4452773 (110 V) Applied Biosystems PN 4452774 (220 V)
SOLiD® 4 Analyzer	Applied Biosystems PN 4452775
SOLiD® 3 Plus to SOLiD®4 Upgrade Kit SOLiD® 3 to SOLiD®4 Upgrade Kit	Applied Biosystems PN 4452784 Applied Biosystems PN 4452785
SOLiD® Light Source	Applied Biosystems PN 4383441
SOLiD® Slide Storage Chamber	Applied Biosystems PN 4406354
SOLiD® Deposition Chambers, 1-Well‡	Applied Biosystems PN 4406352
SOLiD® Deposition Chambers, 4-Well§	Applied Biosystems PN 4406358
SOLiD® Deposition Chambers, 8-Well§	Applied Biosystems PN 4406359
SOLiD® Uninterruptible Power Supply (UPS)	Applied Biosystems PN 4397781 (SOLiD® UPS North America) Applied Biosystems PN 4393695 (220 V; SOLiD® UPS International)
SOLiD® Accessory Disk Drive	Applied Biosystems PN 4426101
SOLiD® Bead Concentration Chart	Applied Biosystems PN 4415131

Item†	Source
Covaris® S2 System (110 V for U.S. customers) (220 V for international customers) The system includes: • Covaris® S2 sonicator • Latitude™ laptop from Dell • MultiTemp III Thermostatic Circulator • Covaris-2 series Machine Holder for (one) 1.5-mL microcentrifuge tube • Covaris-2 series Machine Holder for (one) 0.65-mL microcentrifuge tube • Covaris-2 series Machine Holder for (one) 13 mm × 65 mm tube • Covaris-2 Series Machine Holder for (one) microTUBE • Covaris microTUBE Prep Station • Covaris Water Tank Label Kit • Covaris microTubes (1 pack of 25) For system materials summary, refer to "Covaris® S2 System Materials Summary," *Applied Biosystems SOLiD® 4 System Site Preparation Guide*.	Applied Biosystems PN 4387833 (110 V) Applied Biosystems PN 4392718 (220 V) or Covaris
6-Tube Magnetic Stand	Applied Biosystems AM10055
Microcentrifuge 5417R, refrigerated, without rotor	Eppendorf§ 022621807 (120 V/60 Hz) Eppendorf§ 022621840 (230 V/50 Hz)
FA-45-24-11, fixed-angle rotor, 24 × 1.5/2 mL, including aluminum lid, aerosol-tight	Eppendorf§ 022636006
NanoDrop® ND-1000 Spectrophotometer (computer required)	Thermo Scientific ND-1000
Tabletop Centrifuge (for 96-well plate)	Major Laboratory Supplier (MLS)
Vortexer	MLS
Picofuge	MLS
Magnetic stirrer	MLS
Refrigerator (4°C)	MLS
Freezer (–20°C)	MLS
Pipettors, 20 µL	MLS
Pipettors, 200 µL	MLS
Pipettors, 1000 µL	MLS

† Applied Biosystems has validated this protocol using this specific material. Substitution may adversely affect system performance.

‡ The SOLiD® 4 Analyzer is shipped with 2 of each size SOLiD® Deposition Chambers and SOLiD® 4 Slide Carriers. Two SOLiD® Slide Storage Chambers are provided for use with all chambers.

§ Or equivalent but validation of the equipment for library preparation is required.

Required consumables

Table 16 Required consumables: WFA run

Item†	Source
Nuclease-free Water	Applied Biosystems AM9932
ABgene® 96 1.2-mL square-well storage plates	ABgene AB-1127
3-mm adhesive disks	Grace Bio-Labs ST200
Ethylene glycol	American Bioanalytical AB00455-01000
CF-1 Calibration Fluid Kit	Thermo Scientific CF-1
PR-1 Conditioning Kit‡	Thermo Scientific PR-1
1.5-mL LoBind Tubes	Eppendorf 022431021
Kimwipes®	Major Laboratory Supplier (MLS)
Filtered pipettor tips	MLS

† Applied Biosystems has validated this protocol using this specific material. Substitution may adversely affect system performance.

‡ The NanoDrop® Conditioning Kit is useful for "reconditioning" the sample measurement pedestals to a hydrophobic state if they become "unconditioned" (refer to the Nanodrop® user's manual for more information). The PR-1 kit consists of a container of specially formulated polishing compound and a supply of convenient applicators.

Set up and perform a sequencing run

Required Applied Biosystems reagent kits

The following table lists the required Applied Biosystems reagent kits for sequencing run. For part numbers of individual boxes see Figure 23 on page 40.

Table 17 Required Applied Biosystems reagent kits: Sequencing run

Item (part number)	Components	Kit component(s) used in...
SOLiD® ToP Sequencing Kit – Frag. Lib., F3 Tag, MM35 (4449352)	SOLiD® ToP Frag Lib Seq, F3 Tag – Primer A Master Mix 35 SOLiD® ToP Frag Lib Seq, F3 Tag – Primer B Master Mix 35 SOLiD® ToP Frag Lib Seq, F3 Tag – Primer C Master Mix 35 SOLiD® ToP Frag Lib Seq, F3 Tag – Primer D Master Mix 35 SOLiD® ToP Frag Lib Seq, F3 Tag – Primer E Master Mix 35 SOLiD® Mixing Strip Tube with Zinc	Fragment sequencing up to 35 bases

Item (part number)	Components	Kit component(s) used in...
SOLiD® ToP Sequencing Kit – Frag. Lib., F3 Tag, MM50 (4449388)	SOLiD® ToP Frag Lib Seq, F3 Tag – Primer A Master Mix 50 SOLiD® ToP Frag Lib Seq, F3 Tag – Primer B Master Mix 50 SOLiD® ToP Frag Lib Seq, F3 Tag – Primer C Master Mix 50 SOLiD® ToP Frag Lib Seq, F3 Tag – Primer D Master Mix 50 SOLiD® ToP Frag Lib Seq, F3 Tag – Primer E Master Mix 50 SOLiD® Mixing Strip Tube with Zinc	Fragment sequencing up to 50 bases
SOLiD® ToP Paired End Sequencing Kit – DNA Frag. Lib., MM50/35 (4459179)	SOLiD® ToP Sequencing Kit – Frag. Lib., F3 Tag, MM50 SOLiD® ToP Frag Lib Seq, F3 Tag – Primer A Master Mix 50 SOLiD® ToP Frag Lib Seq, F3 Tag – Primer B Master Mix 50 SOLiD® ToP Frag Lib Seq, F3 Tag – Primer C Master Mix 50 SOLiD® ToP Frag Lib Seq, F3 Tag – Primer D Master Mix 50 SOLiD® ToP Frag Lib Seq, F3 Tag – Primer E Master Mix 50 SOLiD® Mixing Strip Tube with Zinc SOLiD® ToP Sequencing Kit – Frag. Lib., F5-P2 Tag, MM35 SOLiD® ToP Frag Lib Seq, F5-P2 Tag – Primer A Master Mix 35 SOLiD® ToP Frag Lib Seq, F5-P2 Tag – Primer B Master Mix 35 SOLiD® ToP Frag Lib Seq, F5-P2 Tag – Primer C Master Mix 35 SOLiD® ToP Frag Lib Seq, F5-P2 Tag – Primer D Master Mix 35 SOLiD® ToP Frag Lib Seq, F5-P2 Tag – Primer E Master Mix 35 SOLiD® Mixing Strip Tube	Paired-end sequencing of fragment libraries up to 50 bases for the forward read and 35 bases for the reverse read
SOLiD® ToP Paired End Sequencing Kit – RNA Frag. Lib., MM50/35 (4459180)	SOLiD® ToP Sequencing Kit – Frag. Lib., F3 Tag, MM50 SOLiD® ToP Frag Lib Seq, F3 Tag – Primer A Master Mix 50 SOLiD® ToP Frag Lib Seq, F3 Tag – Primer B Master Mix 50 SOLiD® ToP Frag Lib Seq, F3 Tag – Primer C Master Mix 50 SOLiD® ToP Frag Lib Seq, F3 Tag – Primer D Master Mix 50 SOLiD® ToP Frag Lib Seq, F3 Tag – Primer E Master Mix 50 SOLiD® Mixing Strip Tube with Zinc SOLiD® ToP Sequencing Kit – Frag. Lib., F5-BC Tag, MM35 SOLiD® ToP Frag Lib Seq, F5-BC Tag – Primer A Master Mix 35 SOLiD® ToP Frag Lib Seq, F5-BC Tag – Primer B Master Mix 35 SOLiD® ToP Frag Lib Seq, F5-BC Tag – Primer C Master Mix 35 SOLiD® ToP Frag Lib Seq, F5-BC Tag – Primer D Master Mix 35 SOLiD® ToP Frag Lib Seq, F5-BC Tag – Primer E Master Mix 35 SOLiD® Mixing Strip Tube	Paired-end sequencing of RNA libraries up to 50 bases for the forward read and 35 bases for the reverse read

Item (part number)	Components	Kit component(s) used in...
SOLiD® ToP Mate-Paired Sequencing Kit – M-P. Lib., MM35/35 (4452684)	SOLiD® ToP Sequencing Kit – M-P. Lib., F3 Tag, MM35 SOLiD® ToP MP Lib Seq, F3 Tag – Primer A Master Mix 35 SOLiD® ToP MP Lib Seq, F3 Tag – Primer B Master Mix 35 SOLiD® ToP MP Lib Seq, F3 Tag – Primer C Master Mix 35 SOLiD® ToP MP Lib Seq, F3 Tag – Primer D Master Mix 35 SOLiD® ToP MP Lib Seq, F3 Tag – Primer E Master Mix 35 SOLiD® Mixing Strip Tube with Zinc SOLiD® ToP Sequencing Kit – M-P. Lib., R3 Tag, MM35 SOLiD® ToP MP Lib Seq, R3 Tag – Primer A Master Mix 35 SOLiD® ToP MP Lib Seq, R3 Tag – Primer B Master Mix 35 SOLiD® ToP MP Lib Seq, R3 Tag – Primer C Master Mix 35 SOLiD® ToP MP Lib Seq, R3 Tag – Primer D Master Mix 35 SOLiD® ToP MP Lib Seq, R3 Tag – Primer E Master Mix 35 SOLiD® Mixing Strip Tube with Zinc	Mate-pair sequencing up to 35 bases for each tag
SOLiD® ToP Mate-Paired Sequencing Kit – M-P. Lib., MM50/50 (4452685)	SOLiD® ToP Sequencing Kit – M-P. Lib., F3 Tag, MM50 SOLiD® ToP MP Lib Seq, F3 Tag – Primer A Master Mix 50 SOLiD® ToP MP Lib Seq, F3 Tag – Primer B Master Mix 50 SOLiD® ToP MP Lib Seq, F3 Tag – Primer C Master Mix 50 SOLiD® ToP MP Lib Seq, F3 Tag – Primer D Master Mix 50 SOLiD® ToP MP Lib Seq, F3 Tag – Primer E Master Mix 50 SOLiD® Mixing Strip Tube with Zinc SOLiD® ToP Sequencing Kit – M-P. Lib., R3 Tag, MM50 SOLiD® ToP MP Lib Seq, R3 Tag – Primer A Master Mix 50 SOLiD® ToP MP Lib Seq, R3 Tag – Primer B Master Mix 50 SOLiD® ToP MP Lib Seq, R3 Tag – Primer C Master Mix 50 SOLiD® ToP MP Lib Seq, R3 Tag – Primer D Master Mix 50 SOLiD® ToP MP Lib Seq, R3 Tag – Primer E Master Mix 50 SOLiD® Mixing Strip Tube with Zinc	Mate-pair sequencing up to 50 bases for each tag
SOLiD® ToP Fragment BC Sequencing Kit – BC Frag Lib., MM35/5 (4452696)	SOLiD® ToP Sequencing Kit – Frag. Lib., F3 Tag, MM35 SOLiD® ToP Frag Lib Seq, F3 Tag – Primer A Master Mix 35 SOLiD® ToP Frag Lib Seq, F3 Tag – Primer B Master Mix 35 SOLiD® ToP Frag Lib Seq, F3 Tag – Primer C Master Mix 35 SOLiD® ToP Frag Lib Seq, F3 Tag – Primer D Master Mix 35 SOLiD® ToP Frag Lib Seq, F3 Tag – Primer E Master Mix 35 SOLiD® Mixing Strip Tube with Zinc SOLiD® ToP Sequencing Kit – BC Frag. Lib., BC Tag, MM5 Reset Buffer	Multiplex sequencing up to 35 bases for the F3 tag of barcoded fragment libraries (Barcodes 1–16)

Item (part number)	Components	Kit component(s) used in...
SOLiD® ToP Fragment BC Sequencing Kit – BC Frag Lib., MM35/10 (4452698)	SOLiD® ToP Sequencing Kit – Frag. Lib., F3 Tag, MM35 SOLiD® ToP Frag Lib Seq, F3 Tag – Primer A Master Mix 35 SOLiD® ToP Frag Lib Seq, F3 Tag – Primer B Master Mix 35 SOLiD® ToP Frag Lib Seq, F3 Tag – Primer C Master Mix 35 SOLiD® ToP Frag Lib Seq, F3 Tag – Primer D Master Mix 35 SOLiD® ToP Frag Lib Seq, F3 Tag – Primer E Master Mix 35 SOLiD® Mixing Strip Tube with Zinc SOLiD® ToP Sequencing Kit – BC Frag. Lib., BC Tag, MM10 Reset Buffer	Multiplex sequencing up to 35 bases for the F3 tag of barcoded fragment libraries (Barcodes 1–96)
SOLiD® ToP Fragment BC Sequencing Kit – BC Frag Lib., MM50/5 (4452697)	SOLiD® ToP Sequencing Kit – Frag. Lib., F3 Tag, MM50 SOLiD® ToP Frag Lib Seq, F3 Tag – Primer A Master Mix 50 SOLiD® ToP Frag Lib Seq, F3 Tag – Primer B Master Mix 50 SOLiD® ToP Frag Lib Seq, F3 Tag – Primer C Master Mix 50 SOLiD® ToP Frag Lib Seq, F3 Tag – Primer D Master Mix 50 SOLiD® ToP Frag Lib Seq, F3 Tag – Primer E Master Mix 50 SOLiD® Mixing Strip Tube with Zinc SOLiD® ToP Sequencing Kit – BC Frag. Lib., BC Tag, MM5 Reset Buffer	Multiplex sequencing up to 50 bases for the F3 tag of barcoded fragment libraries (Barcodes 1–16)
SOLiD® ToP Fragment BC Sequencing Kit – BC Frag Lib., MM50/10 (4452699)	SOLiD® ToP Sequencing Kit – Frag. Lib., F3 Tag, MM50 SOLiD® ToP Frag Lib Seq, F3 Tag – Primer A Master Mix 50 SOLiD® ToP Frag Lib Seq, F3 Tag – Primer B Master Mix 50 SOLiD® ToP Frag Lib Seq, F3 Tag – Primer C Master Mix 50 SOLiD® ToP Frag Lib Seq, F3 Tag – Primer D Master Mix 50 SOLiD® ToP Frag Lib Seq, F3 Tag – Primer E Master Mix 50 SOLiD® Mixing Strip Tube with Zinc SOLiD® ToP Sequencing Kit – BC Frag. Lib., BC Tag, MM10 Reset Buffer	Multiplex sequencing up to 50 bases for the F3 tag of barcoded fragment libraries (Barcodes 1–96)

Item (part number)	Components	Kit component(s) used in...
SOLiD® ToP Paired End Sequencing Kit – BC Frag. Lib., MM50/35/5 (4459181)	SOLiD® ToP Sequencing Kit – Frag. Lib., F3 Tag, MM50 SOLiD® ToP Frag Lib Seq, F3 Tag – Primer A Master Mix 50 SOLiD® ToP Frag Lib Seq, F3 Tag – Primer B Master Mix 50 SOLiD® ToP Frag Lib Seq, F3 Tag – Primer C Master Mix 50 SOLiD® ToP Frag Lib Seq, F3 Tag – Primer D Master Mix 50 SOLiD® ToP Frag Lib Seq, F3 Tag – Primer E Master Mix 50 SOLiD® Mixing Strip Tube with Zinc SOLiD® ToP Sequencing Kit – BC Frag. Lib., F5-BC Tag, MM35 SOLiD® ToP Frag Lib Seq, F5-BC Tag – Primer A Master Mix 35 SOLiD® ToP Frag Lib Seq, F5-BC Tag – Primer B Master Mix 35 SOLiD® ToP Frag Lib Seq, F5-BC Tag – Primer C Master Mix 35 SOLiD® ToP Frag Lib Seq, F5-BC Tag – Primer D Master Mix 35 SOLiD® ToP Frag Lib Seq, F5-BC Tag – Primer E Master Mix 35 SOLiD® Mixing Strip Tube SOLiD® ToP Sequencing Kit – BC Frag. Lib., BC Tag, MM5 Reset Buffer	Multiplex paired-end sequencing up to 50 bases for the F3 tag of barcoded fragment libraries (Barcodes 1–16)
SOLiD® ToP Paired End Sequencing Kit – BC Frag. Lib., MM50/35/10 (4459182)	SOLiD® ToP Sequencing Kit – Frag. Lib., F3 Tag, MM50 SOLiD® ToP Frag Lib Seq, F3 Tag – Primer A Master Mix 50 SOLiD® ToP Frag Lib Seq, F3 Tag – Primer B Master Mix 50 SOLiD® ToP Frag Lib Seq, F3 Tag – Primer C Master Mix 50 SOLiD® ToP Frag Lib Seq, F3 Tag – Primer D Master Mix 50 SOLiD® ToP Frag Lib Seq, F3 Tag – Primer E Master Mix 50 SOLiD® Mixing Strip Tube with Zinc SOLiD® ToP Sequencing Kit – BC Frag. Lib., F5-BC Tag, MM35 SOLiD® ToP Frag Lib Seq, F5-BC Tag – Primer A Master Mix 35 SOLiD® ToP Frag Lib Seq, F5-BC Tag – Primer B Master Mix 35 SOLiD® ToP Frag Lib Seq, F5-BC Tag – Primer C Master Mix 35 SOLiD® ToP Frag Lib Seq, F5-BC Tag – Primer D Master Mix 35 SOLiD® ToP Frag Lib Seq, F5-BC Tag – Primer E Master Mix 35 SOLiD® Mixing Strip Tube SOLiD® ToP Sequencing Kit – BC Frag. Lib., BC Tag, MM10 Reset Buffer	Multiplex paired-end sequencing up to 50 bases for the F3 tag of barcoded fragment libraries (Barcodes 1–96)

Item (part number)	Components	Kit component(s) used in...
SOLiD® ToP Instrument Buffer Kit (4452688)	Reset Buffer Glycerol 10X Instrument Buffer Cleave Solution 1 Cleave 2.1 Kit (Cleave 2.1 Parts 1 and 2) Storage Buffer Imaging Buffer Kit (Imaging Buffer Parts 1 and 2) 1X T4 Ligase Buffer Kit (Ligase Buffer Parts 1 and 2) Universal Buffer Kit (Universal Buffer Parts 1 and 2)	Sequencing and/or WFA
SOLiD® Flowcell O-rings (4398217)	10-pack of flowcell O-rings	Sequencing and/or WFA
SOLiD® XD Slide & Deposition Kit v2 (4456997)	4 × 2 Sequencing Slides Slide Deposition Buffer v2 Slide Storage Buffer	Bead deposition
Other components	SOLiD® Mixing Strip Tube with Zinc (4449506) SOLiD® Mixing Strip Tube (4406595)	

Required equipment

Table 18 Required equipment: Sequencing run

Item†	Source
SOLiD® 4 System	Applied Biosystems PN 4452773 (110 V) Applied Biosystems PN 4452774 (220 V)
SOLiD® 4 Analyzer	Applied Biosystems PN 4444317
SOLiD® 3 Plus to SOLiD®4 Upgrade Kit SOLiD® 3 to SOLiD®4 Upgrade Kit	Applied Biosystems PN 4452784 Applied Biosystems PN 4452785
SOLiD® Light Source	Applied Biosystems PN 4388441
SOLiD® Slide Storage Chamber	Applied Biosystems PN 4406354
SOLiD® Deposition Chambers, 1-Well‡	Applied Biosystems PN 4406352
SOLiD® Deposition Chambers, 4-Well§	Applied Biosystems PN 4406358
SOLiD® Deposition Chambers, 8-Well	Applied Biosystems PN 4406359
SOLiD® 4 Slide Carriers§	Applied Biosystems PN 4453027

Item†	Source
SOLiD® Uninterruptible Power Supply (UPS)	Applied Biosystems PN 4397781 (SOLiD® UPS North America) Applied Biosystems PN 4393695 (220 V; SOLiD® UPS International)
SOLiD® Accessory Disk Drive	Applied Biosystems PN 4426101
SOLiD® Bead Concentration Chart	Applied Biosystems PN 4415131
Covaris® S2 System (110 V for U.S. customers) (220 V for international customers) The system includes: • Covaris® S2 sonicator • Latitude™ laptop from Dell • MultiTemp III Thermostatic Circulator • Covaris-2 series Machine Holder for (one) 1.5-mL microcentrifuge tube • Covaris-2 series Machine Holder for (one) 0.65-mL microcentrifuge tube • Covaris-2 series Machine Holder for (one) 13 mm × 65 mm tube • Covaris-2 Series Machine Holder for (one) microTube • Covaris microTube Prep Station • Covaris Water Tank Label Kit • Covaris microTubes (1 pack of 25) For system materials summary, refer to "Covaris® S2 System Materials Summary," *Applied Biosystems SOLiD® 4 System Site Preparation Guide*.	Applied Biosystems PN 4387833 (110 V) Applied Biosystems PN 4392718 (220 V) or Covaris
6-Tube Magnetic Stand	Applied Biosystems AM10055
Microcentrifuge 5417R, refrigerated, without rotor	Eppendorf§ 022621807 (120 V/60 Hz) Eppendorf§ 022621840 (230 V/50 Hz)
FA-45-24-11, fixed-angle rotor, 24 × 1.5/2 mL, including aluminum lid, aerosol-tight	Eppendorf§ 022636006
NanoDrop® ND-1000 Spectrophotometer (computer required)	Thermo Scientific ND-1000
Tabletop Centrifuge (for 96-well plate)	Major Laboratory Supplier (MLS)
Vortexer	MLS
Picofuge	MLS
Magnetic stirrer	MLS
Refrigerator (4°C)	MLS
Freezer (–20°C)	MLS
Pipettors, 20 µL	MLS
Pipettors, 200 µL	MLS
Pipettors, 1000 µL	MLS

† Applied Biosystems has validated this protocol using this specific material. Substitution may adversely affect system performance.

‡ The SOLiD® 4 Analyzer is shipped with 2 of each size SOLiD® Deposition Chambers and SOLiD® 4 Slide Carriers. Two SOLiD® Slide Storage Chambers are provided for use with all chambers.

§ Or equivalent but validation of the equipment for library preparation is required.

Required consumables

Table 19 Required consumables: Sequencing run

Item†	Source
Nuclease-free Water	Applied Biosystems AM9932
ABgene® 96 1.2-mL square-well storage plates	ABgene AB-1127
3-mm adhesive disks	Grace Bio-Labs ST200
Ethylene glycol	American Bioanalytical AB00455-01000
CF-1 Calibration Fluid Kit	Thermo Scientific CF-1
PR-1 Conditioning Kit‡	Thermo Scientific PR-1
1.5-mL LoBind Tubes	Eppendorf 022431021
Kimwipes®	Major Laboratory Supplier (MLS)
Filtered pipettor tips	MLS

† Applied Biosystems has validated this protocol using this specific material. Substitution may adversely affect system performance.

‡ The NanoDrop® Conditioning Kit is useful for "reconditioning" the sample measurement pedestals to a hydrophobic state if they become "unconditioned" (refer to the Nanodrop® user's manual for more information). The PR-1 kit consists of a container of specially formulated polishing compound and a supply of convenient applicators.

B Supplemental Procedures

This appendix covers:

- Clean the deposition chamber 95
- Calibrate the deposition chamber volume 96
- Clean the Instrument Buffer bottle 97
- Install the SOLiD® System Flowcell O-ring 102
- Clean the reagent strip cover 103
- Replace the SOLiD® Light Source 104
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- Manually find the focus range 108
- Shut down the SOLiD® 4 Analyzer 119
- Reset the robot position 119
- Store the slide in a flowcell 119
- Modify the Barcode Error Correction Level 120

Clean the deposition chamber

The enhanced surface chemistry on the SOLiD® XD Slides is extremely robust and serves to efficiently bind templated beads, but it is also extremely sensitive to contaminants. Contaminants will result in a decrease in bead binding if the proper protocol is not followed. In addition, use only supported consumables and reagents for bead preparation and deposition.

Common sources of contamination include:

- Improper cleaning of deposition chambers.
- Incomplete drying of the deposition chamber.
- Inadequate buffer exchanges and washes in bead samples.

It is critical that the user follows the proper procedure for cleaning and preparation of the deposition chamber. Follow the proceeding instructions:

1. Clean the deposition chamber by washing thoroughly in hot water for at least 1 minute.

2. Then, thoroughly rinse with DI water for 2 minutes.

3. Dry chambers completely using lint-free wipes (i.e., Kimwipes®) or air-dry overnight at room temperature.

4. Do not put the chambers in 37°C to dry, as this allows any residual buffer/ contaminants from the gasket to seep out, and will adversely affect your deposition.

5. Do not dry with compressed air because any contaminants in the air system will affect the deposition.

Deposition chambers previously used with the SOLiD® 3 or SOLiD® 3 Plus System

For users who have upgraded from the SOLiD® 3 or SOLiD® 3 Plus System to the SOLiD® 4 System, and who are thus still using the same deposition chambers, it is recommended that they stop using detergents to clean the deposition chamber, since the SOLiD® XD Slides are highly sensitive to any detergent contamination. For deposition chambers used before with the SOLiD® 3 or SOLiD® 3 Plus System, an additional cleaning protocol (outlined below), should be conducted:

1. Rinse the deposition chamber top, bottom, and carrier thoroughly with hot water for at least 1 minute.

2. Fill a sonicator, capable of holding the deposition chamber top, with DI water to the appropriate level. (No additives should be added to DI water used for sonication).

3. Place only the deposition chamber top into the sonicator, and sonicate at a medium-low setting for 10 minutes.

4. Remove the sonicated deposition chamber top, and thoroughly rinse with DI water for 2 minutes.

5. Rinse the deposition chamber bottom and carrier with DI water for 2 minutes.

6. Dry the chambers thoroughly with lint-free wipes, or allow the chambers to air-dry, at room temperature, overnight.

Calibrate the deposition chamber volume

The proper filling of the deposition chamber is crucial for uniform depositions:

- Underestimation of the chamber volume can cause bubbles to remain in the chamber during centrifugation and result in bead loss and uneven depositions.

- Overestimation of chamber volume will lead to decreased densities due to beads remaining in the pipette.

To properly calibrate the volume of the deposition chamber, follow the protocol:

1. Obtain a new or used SOLiD® XD Slide.

2. Assemble the deposition chamber with the SOLiD® XD Slide.

3. Pipet a 1 mL of DI water into the deposition chamber assembly. Once the chamber is full, adjust the pipette until the remaining water is at the end of the pipette tip.

4. Subtract this measurement from the 1 mL to yield the chamber volume.

5. Record the deposition chamber volume measurement.

6. Disassemble the deposition chamber, and dry the chamber and the slide with a lint-free wipe. Reassemble after the chamber after drying is complete.

7. Repeat steps 2–4 twice, for a total of 3 volume measurements.

8. Take the average volume from the three measurements.

9. Clean the deposition chamber thoroughly after the calibration is complete.

10. Measure the exact volume of each chamber using this technique.

11. Mark each chamber with exact volume as to best support correct loading procedures.

Bubbles during deposition or sequencing

- Significant bubbles during slide loading or during sequencing can cause large regions of bead loss across the slide.
- Ensure that the instrument buffer lines are properly primed prior to slide loading.

Clean the Instrument Buffer bottle

Regular cleaning of the Instrument Buffer bottle is required for every run. Failure to clean the Instrument Buffer bottle regularly may allow microbial contaminants to proliferate in the system.

If the level of Instrument Buffer in the Instrument Buffer bottle falls below the recommended fill volume, do not add new Instrument Buffer to "top off " the Buffer that has been standing in the Instrument Buffer bottle. Topping off can lead to contamination.

Required equipment

Table 20 Required equipment: Clean the Instrument Buffer bottle

Item	Source
Beaker or graduated cylinder	Major Laboratory Supplier (MLS)
Bottle brush	MLS

Table 21 Required consumables: Clean the Instrument Buffer bottle

Item	Source
Deionized water	Major Laboratory Supplier (MLS)†
Bleach	MLS
Water	MLS

† For the SDS of any chemical not distributed by Applied Biosystems, contact the chemical manufacturer. Before handling any chemicals, refer to the SDS provided by the manufacturer, and observe all relevant precautions..

1. Rinse the Instrument Buffer bottle three times with approximately 1 L of deionized water.
2. Inspect the Instrument Buffer bottle for visible signs of microbial contaminants.
3. If any contaminants are present, clean the Instrument Buffer bottle with bleach:
   a. Pour approximately 500 mL of bleach into the Instrument Buffer bottle.
   b. Add approximately 500 mL of water to the bleach in the bottle.
   c. Scrub the bottle with a bottle brush.
   d. Rinse the bottle at least two times with water.
   e. Rinse the bottle at least three more times with deionized water.

Clean the air filter

Regular cleaning of the air filter is required once a month. Failure to clean the air filter can cause the filter to clog with dust, which may lead to poor air flow and temperature control for the flowcells. You should clean the filter between runs (but not during runs).

Required consumables

Table 22 Required consumables: Clean the air filter

Item	Source
Kimwipes® wipers	Major Laboratory Supplier (MLS)

1. At the back of the instrument, remove the plastic filter cover with the filter (see Figure 62).

   Figure 62 Remove the plastic filter cover.

2. Separate the plastic cover from the air filter (see Figure 64).

   Figure 63 Separate the plastic cover (left) from the air filter (right).

3. Use a Kimwipes® wiper to clean the filter. If the filter is heavily clogged, rinse the filter under running water and dry thoroughly overnight.
4. Reinstall the filter and plastic filter cover on the instrument.

Flush the fluidic lines

If the SOLiD® 4 Analyzer is in continuous use, the fluidics system in the instrument generally does not need much routine care beyond regular cleaning of the Instrument Buffer bottle and flushing of the lines every three months. However, if the SOLiD® 4 Analyzer will be sitting idle for more than two weeks, flush the fluidic system and power off the instrument with the fluidic lines empty.

Note: The instrument flush procedure comprises two scripts. Each script takes approximately 13 minutes to complete.

Required consumables

Table 23 Required consumables: Flush the fluidic lines

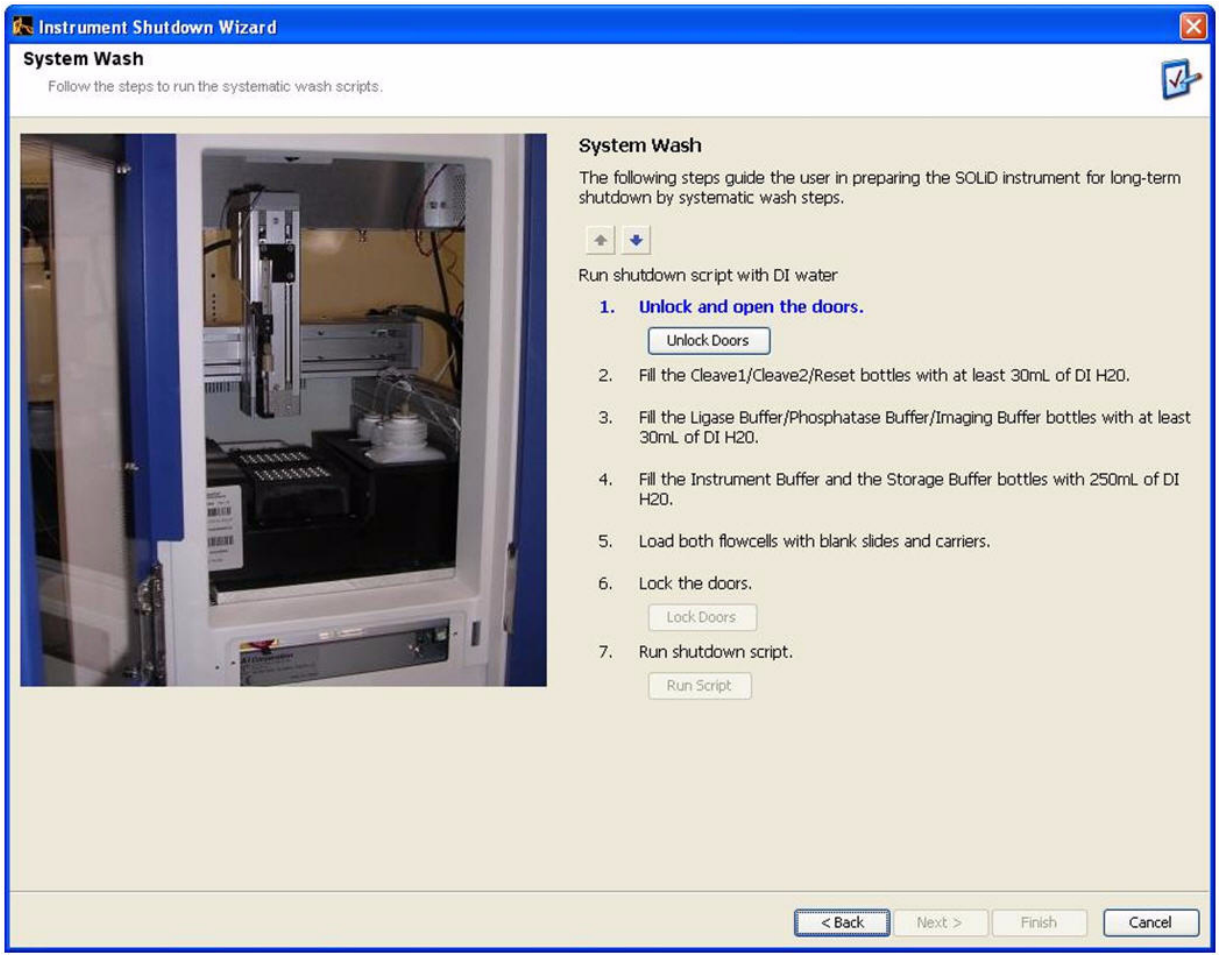
Item	Source
Deionized water	Major Laboratory Supplier (MLS)
Slides	MLS

1. Open the Instrument Shutdown wizard by choosing **Wizards ▸ Instrument Shutdown** (Figure 64).

   Figure 64 Open Instrument Shutdown Wizard.

2. In the Wizard's introduction screen, click **Next**.
3. In the System Wash screen, click **Unlock Doors** (Figure 65).

Figure 65 Complete the actions in the first System Wash screen.


Instrument Shutdown Wizard
System Wash
Follow the steps to run the systematic wash scripts.
System Wash
The following steps guide the user in preparing the SOLiD instrument for long-term shutdown by systematic wash steps.
Run shutdown script with DI water
1. Unlock and open the doors.
Unlock Doors
2. Fill the Cleave1/Cleave2/Reset bottles with at least 30mL of DI H20.
3. Fill the Ligase Buffer/Phosphatase Buffer/Imaging Buffer bottles with at least 30mL of DI H20.
4. Fill the Instrument Buffer and the Storage Buffer bottles with 250mL of DI H20.
5. Load both flowcells with blank slides and carriers.
6. Lock the doors.
Lock Doors
7. Run shutdown script.
Run Script
< Back
Next >
Finish
Cancel

4. Replace all of the bottles from the side of the instrument, the chiller block, and the cabinet with rinsed-out bottles.

   All bottles *except* the Instrument Buffer and Storage Buffer bottles should contain at least 30 mL of deionized water. The Instrument Buffer and Storage Buffer bottles. These two bottles require 250 mL.

5. Load a slide onto each of the flowcells. You can load used slides or 1× 3-inch standard microscope slides for this step.

6. Click **Lock Doors**.

7. Click **Run Script** to begin instrument flush.

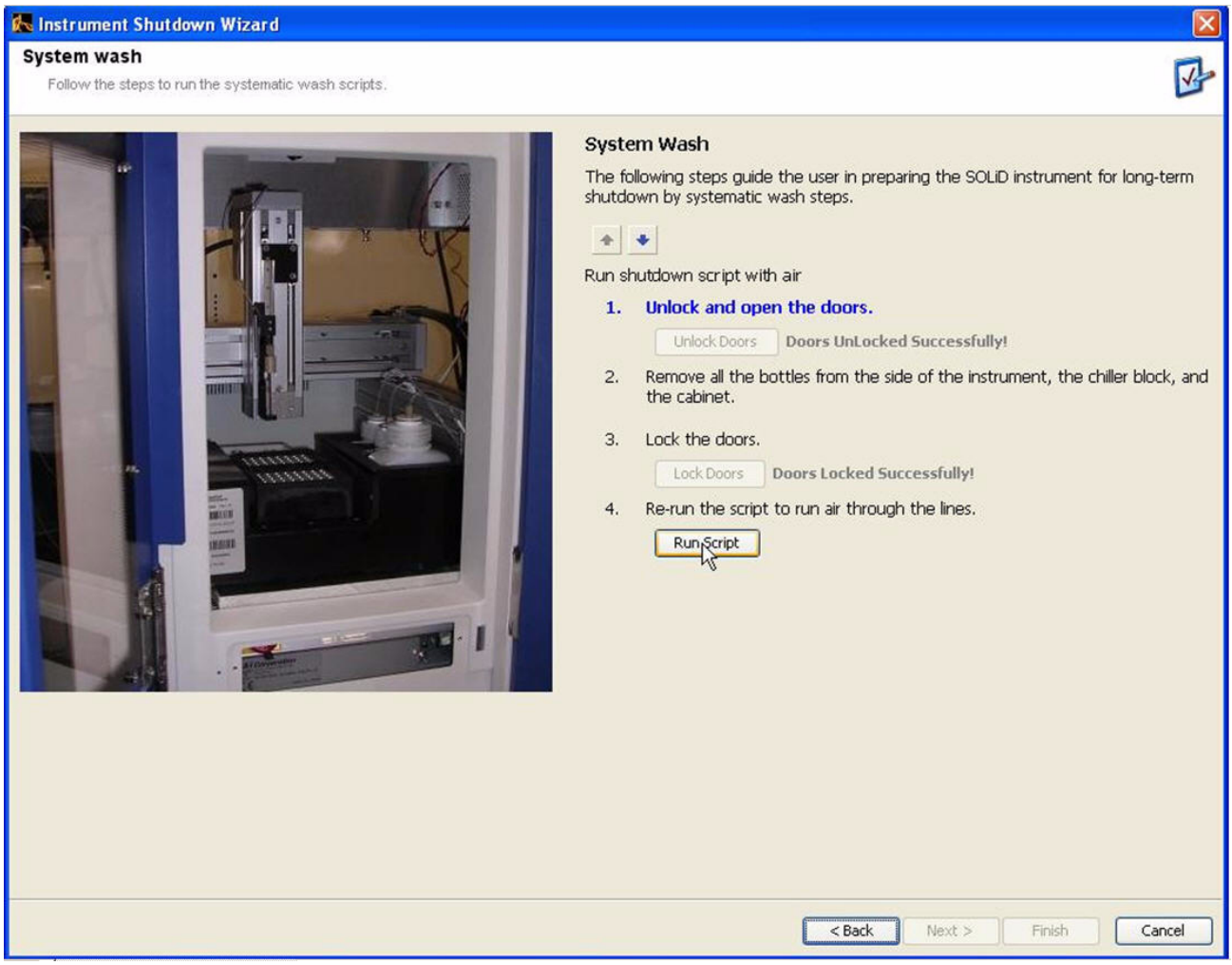
   Note: The script takes approximately 13 minutes to complete.

8. After the script has completed, click **Next**.

9. In the System Wash screen, click **Unlock Doors** and remove all of the bottles from the side of the instrument, the chiller block, and the cabinet.

   You will now perform a dry flush of the system (Figure 66).

Figure 66 Complete the actions in the second System Wash screen.

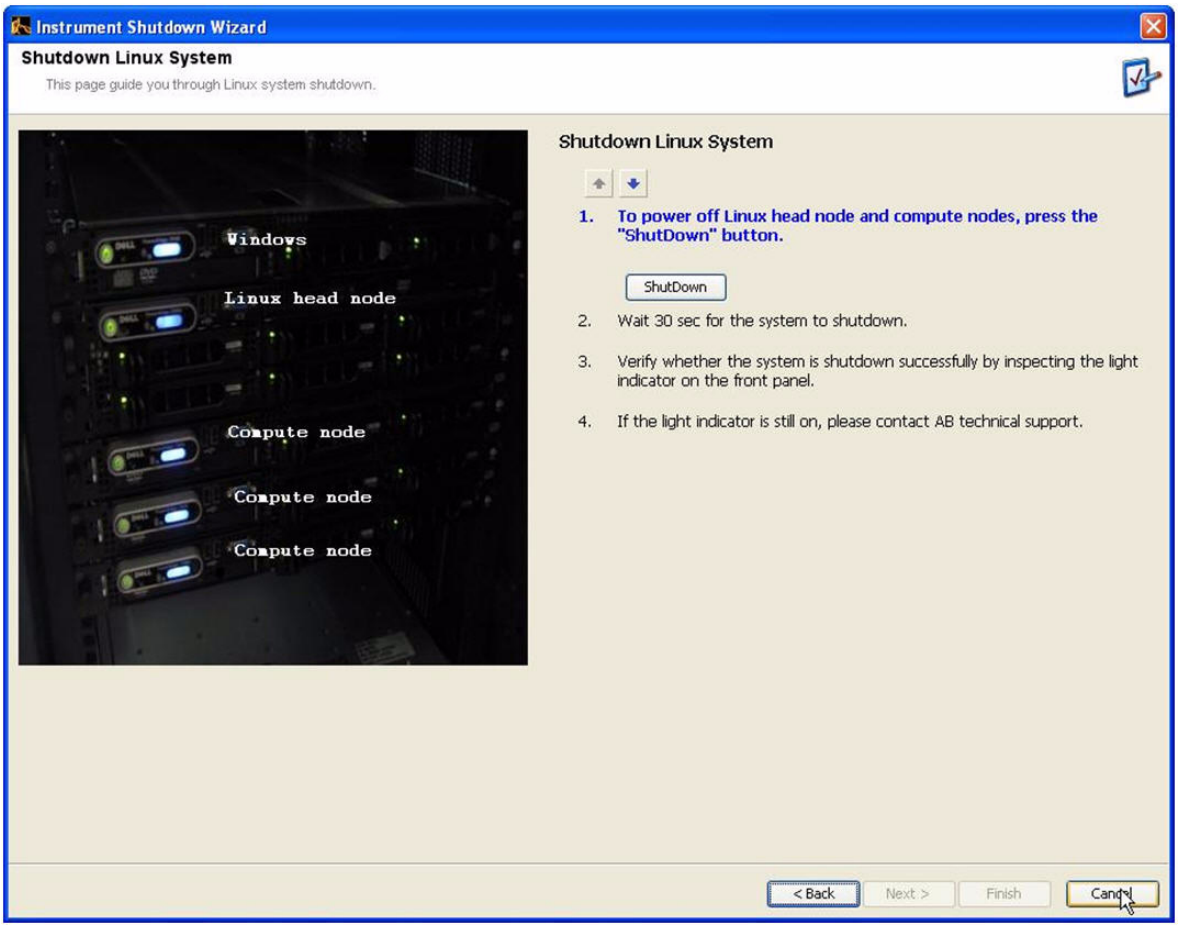

Instrument Shutdown Wizard
System wash
Follow the steps to run the systematic wash scripts.
System Wash
The following steps guide the user in preparing the SOLiD instrument for long-term shutdown by systematic wash steps.
Run shutdown script with air
1. Unlock and open the doors.
Unlock Doors
Doors UnLocked Successfully!
2. Remove all the bottles from the side of the instrument, the chiller block, and the cabinet.
3. Lock the doors.
Lock Doors
Doors Locked Successfully!
4. Re-run the script to run air through the lines.
Run Script
< Back
Next >
Finish
Cancel

10. To begin the dry instrument flush, click **Run Script**.

    Note: This script takes approximately 13 minutes to complete.

11. Perform one of the following:
    - SOLiD® 4 Analyzer **in continuous use**: If the SOLiD® 4 Analyzer is *in continuous use* and you are performing this flush every three months as required for preventive maintenance, click **Cancel** (Figure 67). Do not shut down the power to the Linux head node and compute nodes.
    - SOLiD® 4 Analyzer **idle for more than two weeks**: If the SOLiD® 4 Analyzer *will not be used for more than two weeks*, click **ShutDown** (Figure 67). Close down the SOLiD® Instrument Control Software and power off the instrument.

Figure 67 Click Cancel if performing regular maintenance or ShutDown to power off the instrument.


Instrument Shutdown Wizard
Shutdown Linux System
This page guide you through Linux system shutdown.
Windows
Linux head node
Compute node
Compute node
Compute node
Shutdown Linux System
1. To power off Linux head node and compute nodes, press the "ShutDown" button.
ShutDown
2. Wait 30 sec for the system to shutdown.
3. Verify whether the system is shutdown successfully by inspecting the light indicator on the front panel.
4. If the light indicator is still on, please contact AB technical support.
< Back
Next >
Finish
Cancel

Install the SOLiD® System Flowcell O-ring

After each run, check the SOLiD® System Flowcell O-ring for cuts and abrasions. If you see any abnormalities, replace the O-ring. Inspect the O-ring groove for debris or contamination. Clean the O-ring groove with water as needed.

Required equipment

Table 24 Required equipment: Install the SOLiD® Flowcell O-ring

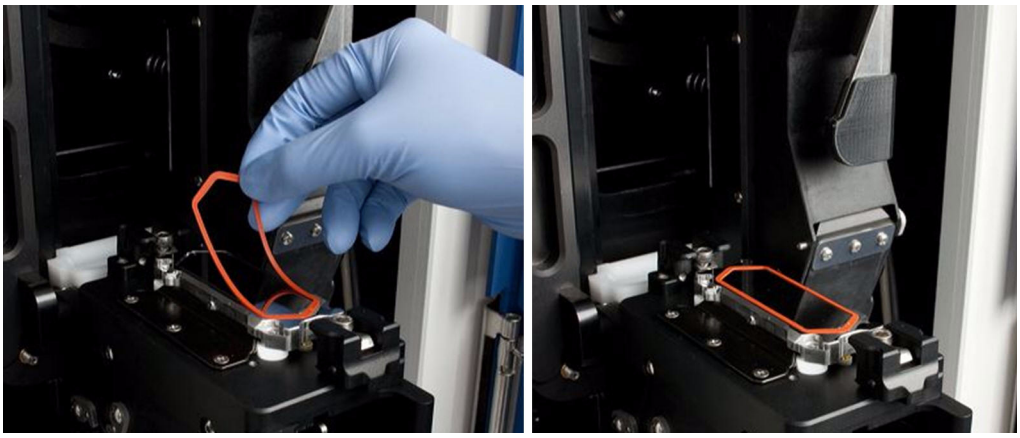
Item	Source
SOLiD® System Flowcell O-ring, 10-pack	Applied Biosystems 4398217

Procedure

Insert the O-ring into the groove on the flowcell so that the smooth side is on top (see Figure 68).

Run your finger around the O-ring to flatten any high spots.

Figure 68 Install the SOLiD® O-Ring on a flowcell.



Clean the reagent strip cover

Inspect the top and bottom of the reagent strip covers after each run for splattered wet or dry reagents. If you see reagent on a cover, clean the cover (see Table 27).

Required equipment

Table 25 Required equipment: Clean the reagent strip cover

Item	Source
Pipette or graduated cylinder	Major Laboratory Supplier (MLS)†
Scrub brush	MLS

† For the SDS of any chemical not distributed by Applied Biosystems, contact the chemical manufacturer. Before handling any chemicals, refer to the SDS provided by the manufacturer, and observe all relevant precautions.

Required consumables

Table 26 Required consumables: Clean the reagent strip cover

Item	Source
Extran 300	VWR EM-EX0996-2
Kimwipes® wipers	Major Laboratory Supplier (MLS)
Deionized water	MLS
Cotton swabs	MLS

Table 27 Clean the reagent strip cover according to location or amount of reagent splatter

If you see...	Then
Minor splashes	Wipe the reagent strip cover with a Kimwipes® wiper. If the reagents are dried, first wet the Kimwipes® wiper moistened with deionized water or a dilution of Extran 300 wash detergent in deionized water.

If you see...	Then
Reagents in the holes of the of the reagent strip cover	Wipe the inside surfaces of the holes with a cotton swab. If the reagents are dried, first wet the cotton swab as necessary with deionized water or a dilution of Extran 300 wash detergent in deionized water.
Large areas of dried reagents	Wash the covers in a dilution of Extran wash detergent (10 mL Extran 300 in 1 L of deionized water). If needed, use a scrub brush.

Replace the SOLiD® Light Source

CAUTION! PHYSICAL INJURY HAZARD. Hot Surface. Surface of the SOLiD® Light Source may be hot. Use care when working around the SOLiD® Light Source to avoid being burned.

Replace the SOLiD® Light Source in the SOLiD® 4 Analyzer every 1500 hours.

Required equipment

Table 28 Replace the SOLiD® Light Source

Item	Source
SOLiD® Light Source	Applied Biosystems 4388441

1. Unscrew the 4 screws retaining the light box access cover on the top of the instrument.
2. Remove the cover to the light source from the housing.
3. Pull the light source straight up and out of the unit.
4. Slide a new SOLiD® Light Source into place. Ensure that the light source is oriented in the correct direction (see Figure 69).
5. Refit and screw down the access cover on the housing.
6. In the System Status menu on the ICS, click **Reset** to reset the lamp timer.

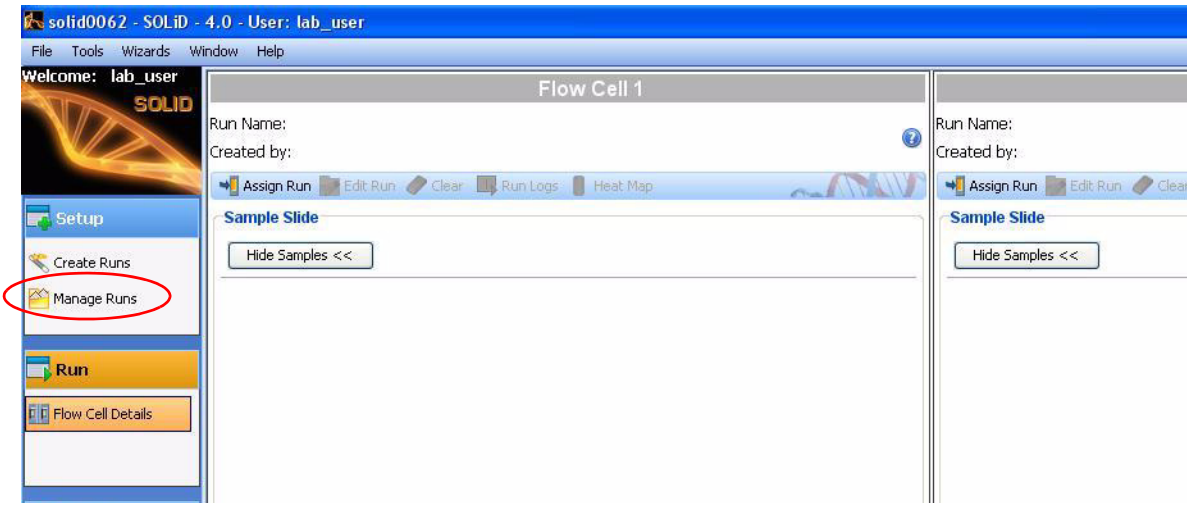
Figure 69 Orientation of the SOLiD® Light Source.

Set up a run by importing a Run Definition file

A sequencing run can be set up by importing a Run Definition file that has been created offline. Setting up a run by importing a run definition file saves time re-entering information of a repeated run.

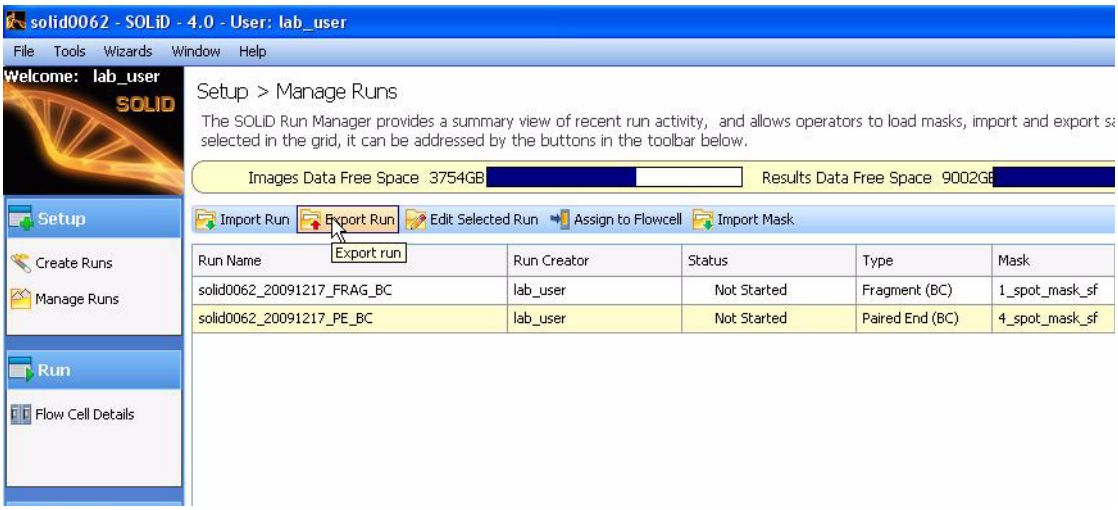
1. Click **Manage Runs** in the task pane on the left menu pane of the ICS (see Figure 70).

Figure 70 Manage Runs.



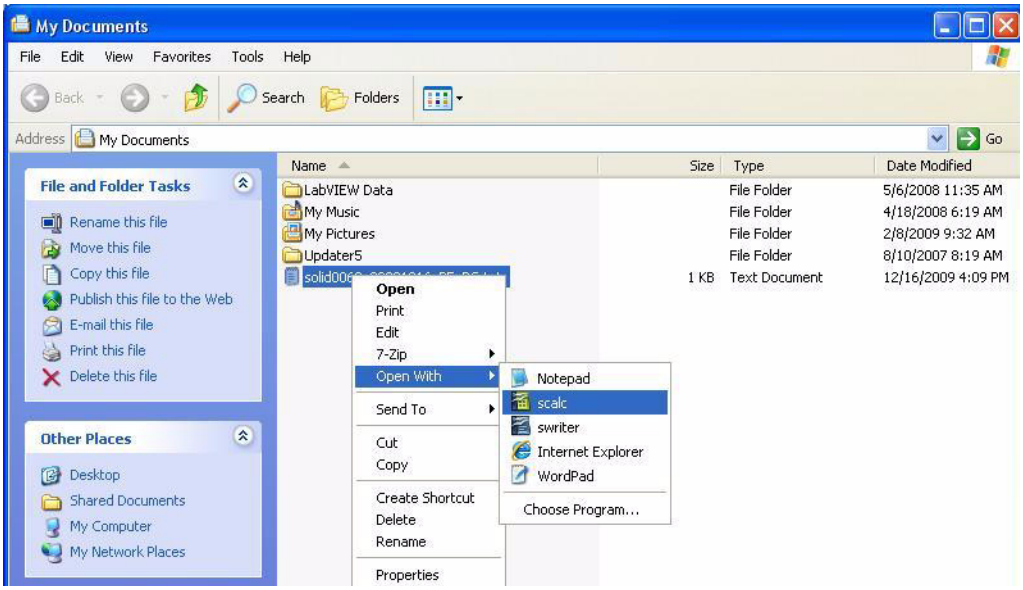
2. Click **Export Run** (see Figure 71).

Figure 71 Export Run.



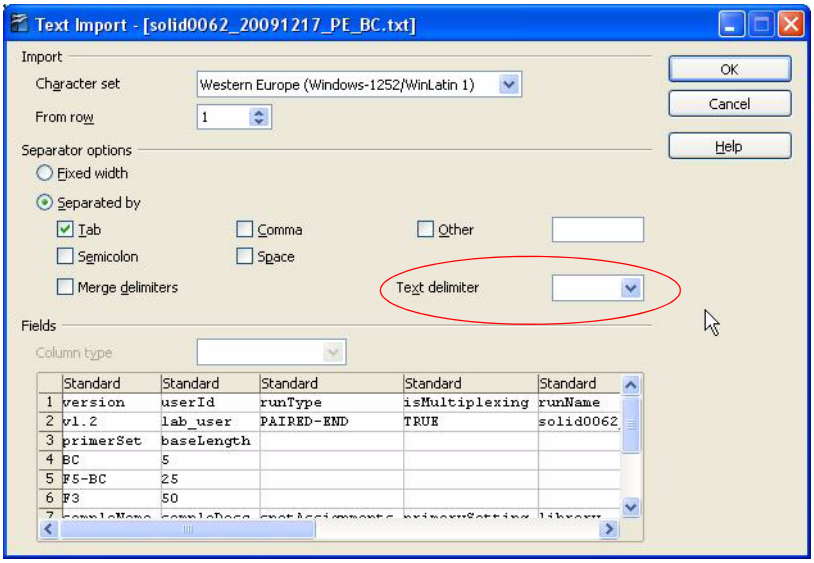
3. Open the Run Definition file that you exported using scalc (see Figure 72). You can use another spreadsheet program.

Figure 72 Open the Run Definition file.



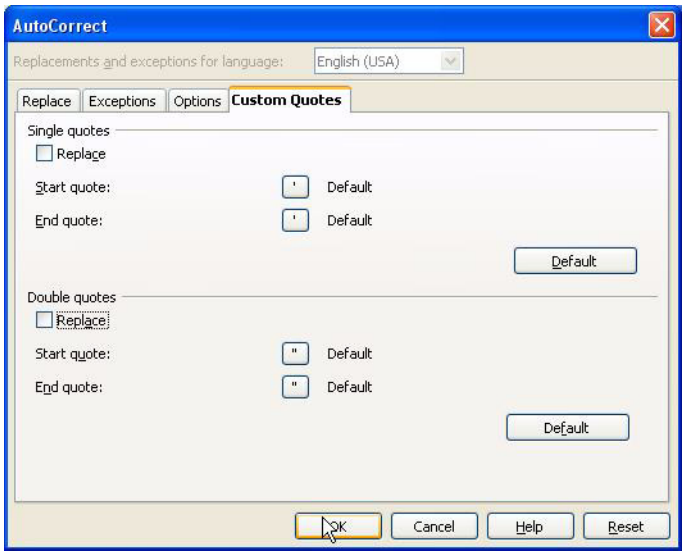
4. In the Text Importer window, empty the Text Delimiter box by clicking in the Text Delimiter box and pressing the **Backspace** key to delete the quotation marks (see Figure 73). Then click OK.

Figure 73 Empty the Text Delimiter box.



5. Select **Tools** ▸ **Autocorrect Options**. In the Custom Quotes tab, uncheck the **Replace** box in the Double Quotes section (see Figure 74). If this box is checked, the file will not be imported correctly into ICS because directional quotation marks will be used instead of non-directional (straight) quotation marks.

Figure 74 Uncheck Replace in the Double quotes section.



6. Finish entering the sample, library, and if applicable, barcode information using the fill down function. You can access this function by selecting **Edit ▸ Fill ▸ Down**. If you assign multiple barcodes to a single library, enter the barcodes in quotes, separated by commas (see Figure 75).

Figure 75 Complete the sample, library, and barcode information.

solid0062_20091217_PE_BC.txt - OpenOffice.org Calc

File Edit View Insert Format Tools Data Window Help

Arial 10

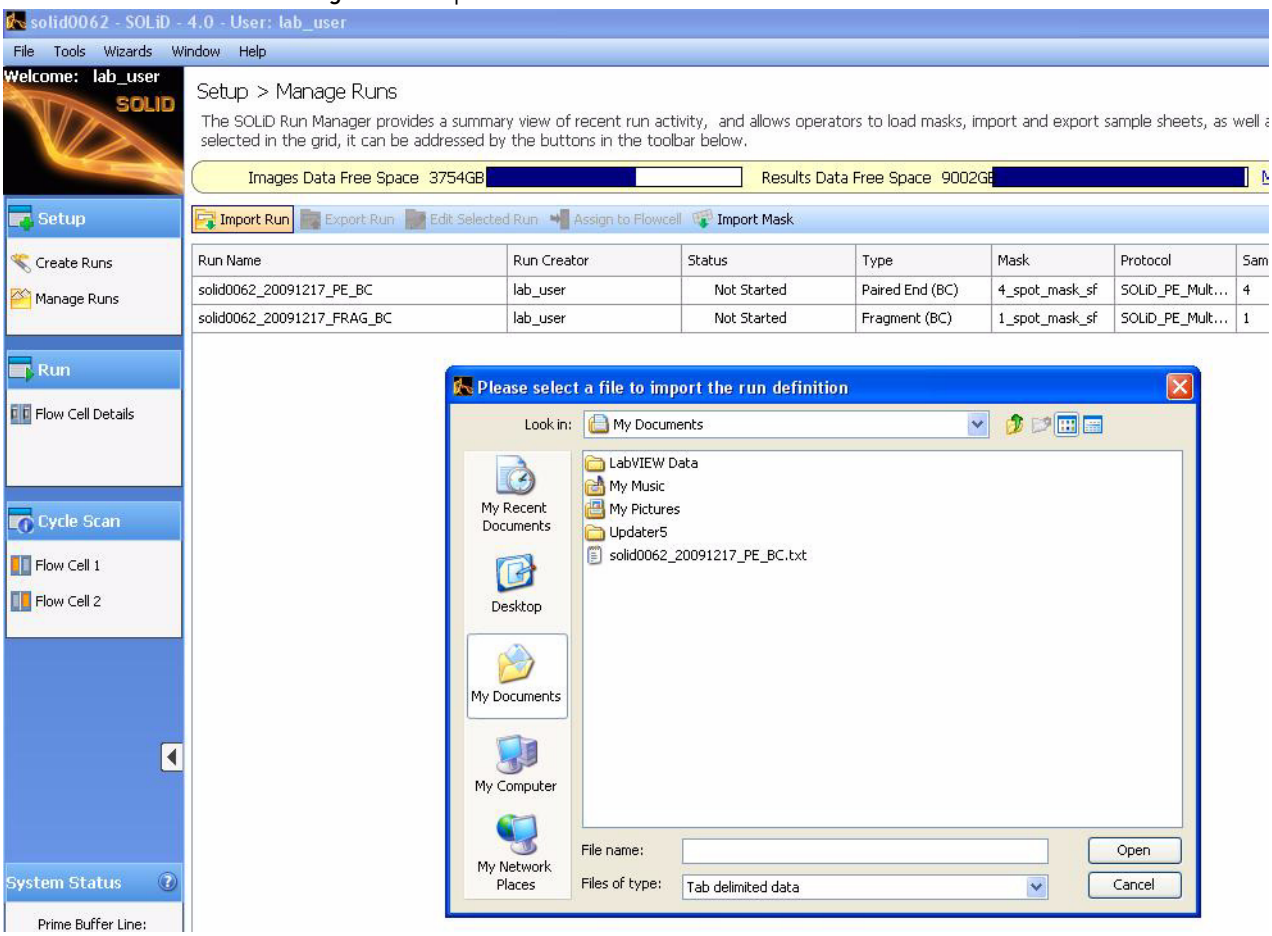
K32

	A	B	C	D	E	F	G	H	I
1	version	userId	runType	isMultiplexing	runName	runDesc	mask	protocol	
2	v1.2	lab_user	PAIRED-END	TRUE	solid0062_20091217_PE_BC		4_spot_mask_sf	SOLiD_PE_Multiplex	
3	primerSet	baseLength							
4	BC	5							
5	F5-BC	25							
6	F3	50							
7	sampleName	sampleDesc	spotAssignments	primarySetting	library	application	multiplexingSeries	barcodes	
8	bcSample1		1	Barcode_1MM	Lib1		BC Kit Module 1-16	"1,2"	
9	bcSample1		1	Barcode_1MM	Lib2		BC Kit Module 1-16	"3,4"	
10	bcSample1		1	Barcode_1MM	Lib3		BC Kit Module 1-16	"5,6"	
11	bcSample2		2	Barcode_1MM	Lib1		BC Kit Module 1-16	1	
12	bcSample2		2	Barcode_1MM	Lib2		BC Kit Module 1-16	2	
13	bcSample2		2	Barcode_1MM	Lib3		BC Kit Module 1-16	3	
14	bcSample2		2	Barcode_1MM	Lib4		BC Kit Module 1-16	4	
15	bcSample2		2	Barcode_1MM	Lib5		BC Kit Module 1-16	5	
16	bcSample2		2	Barcode_1MM	Lib6		BC Kit Module 1-16	6	
17	bcSample2		2	Barcode_1MM	Lib7		BC Kit Module 1-16	7	
18	bcSample2		2	Barcode_1MM	Lib8		BC Kit Module 1-16	8	
19	bcSample2		2	Barcode_1MM	Lib9		BC Kit Module 1-16	9	
20	bcSample2		2	Barcode_1MM	Lib10		BC Kit Module 1-16	10	
21	bcSample2		2	Barcode_1MM	Lib11		BC Kit Module 1-16	11	
22	bcSample2		2	Barcode_1MM	Lib12		BC Kit Module 1-16	12	
23	bcSample2		2	Barcode_1MM	Lib13		BC Kit Module 1-16	13	
24	bcSample2		2	Barcode_1MM	Lib14		BC Kit Module 1-16	14	
25	bcSample2		2	Barcode_1MM	Lib15		BC Kit Module 1-16	15	
26	bcSample2		2	Barcode_1MM	Lib16		BC Kit Module 1-16	16	
27	bcSample3		3	Barcode_1MM	Lib1		BC Kit Module 1-16	"1"	

IMPORTANT! Be sure to review each sample's library and barcode assignment before starting a run. After a run starts, you cannot change the library or barcode assignment.

7. Save the file by selecting **File ▸ Save**. Do not select "Save As," because scalc does not allow files to be saved as .txt files.
8. Click **Keep Current Format**.
9. In ICS, click **Manage Runs**.
10. Click **Import Run** and select the run definition file you modified with scalc (see Figure 76).

Figure 76 Import the run definition file.



11. To assign a run previously saved to the database:
    a. Click on **Manage Runs** in the task pane.
    b. Click the run, then select **Assign to Flowcell**.
    c. Choose a flowcell, then click **OK**.

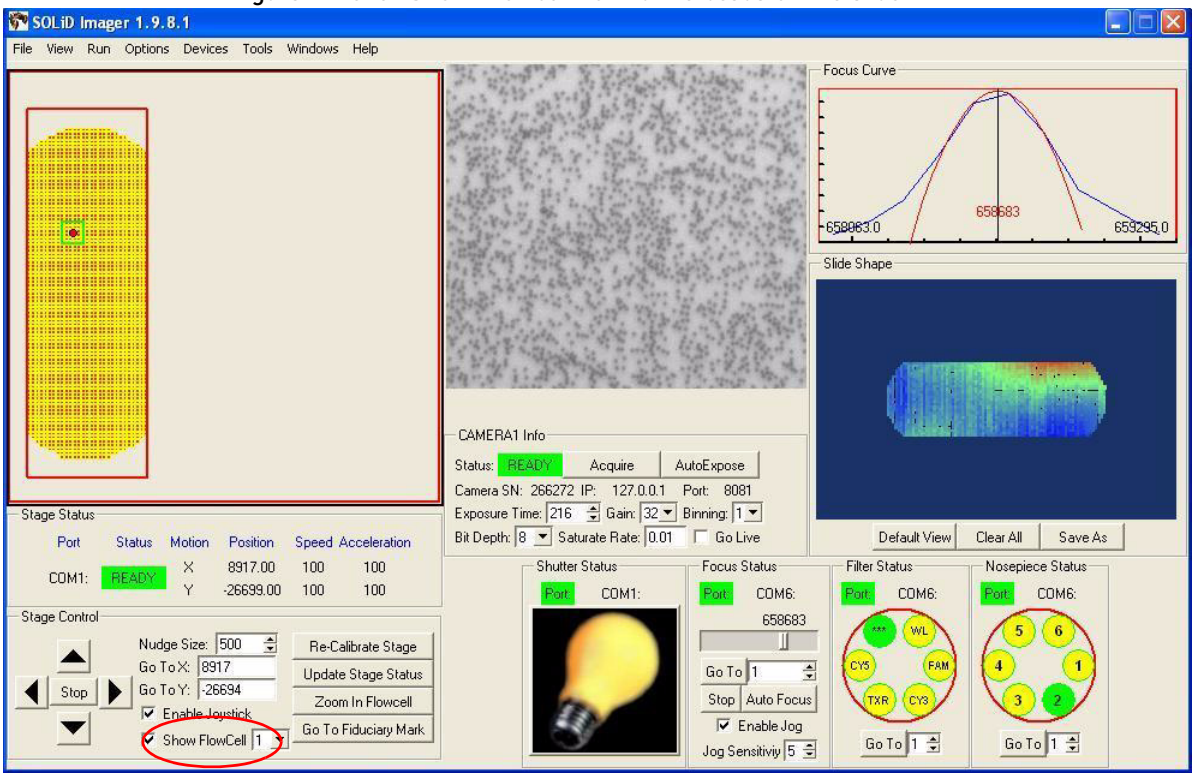
Manually find the focus range

You should first attempt automatic range detection (see Chapter 3, "Detect the focus range" on page 49). If automatic detection fails, then use the manual mode.

Select the flowcell, then find the beads on the slide

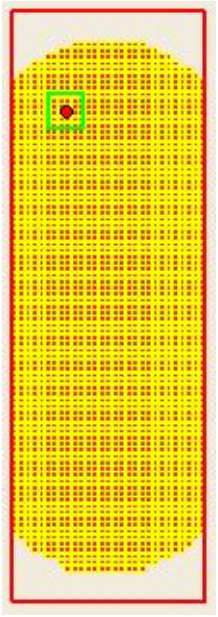
1. Close the front doors of the SOLiD® 4 Analyzer. Open the Imager window by choosing **Window** ▸ **Imaging System**.
2. Select the **Show Flowcell** box in the bottom left hand corner of the Imager window, then choose the number of the flowcell to set (Flowcell **1** or Flowcell **2**; see Figure 77).

Figure 77 Click **Show Flowcell** to find the beads on the slide.



3. From the View menu, choose **Stage Template**, then navigate to the C:\Runs directory. Find the folder in the Runs directory that corresponds to the run just set up, then choose the .STG file for the run. Choosing Stage Template and the .STG file superimposes the slide layout on the Imager screen.
4. Use the mouse to drag the green-box cursor to a position on the upper-left side of the slide. Dragging the green-box cursor moves the flowcell stage to that corner of the slide (see Figure 78 on page 110).

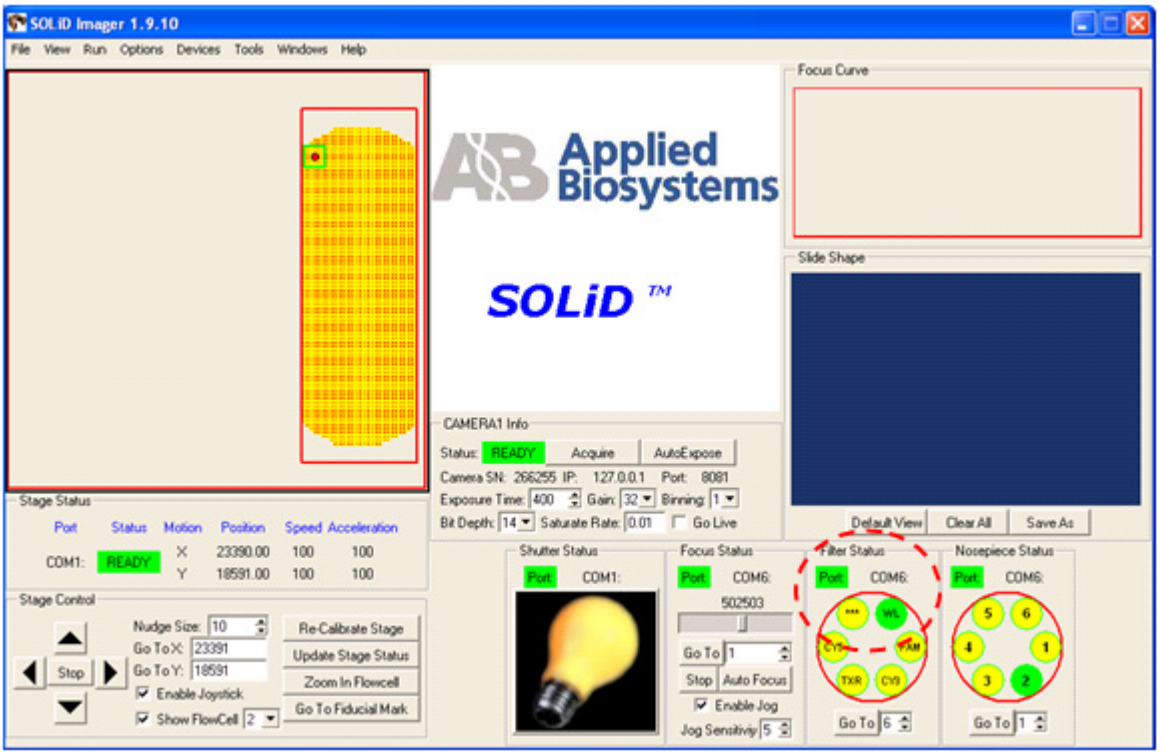
Figure 78 Use the mouse to drag the green-box cursor and to move the flowcell stage the same way.



Find the focal range

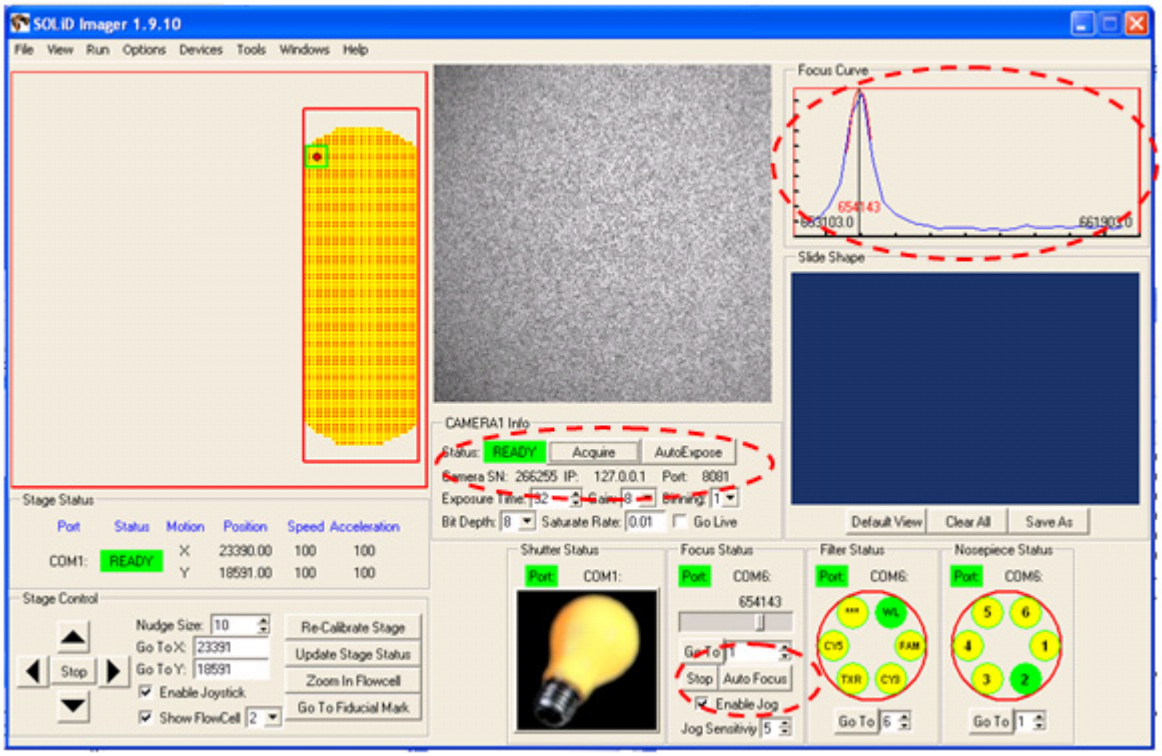
1. Set the Filter Status to **WL** (white light; see Figure 79).

Figure 79 Click **WL** (white light) to set the Filter Status.



2. In the Imager window, click the **AutoFocus** button, then wait about 20 seconds for the Imager to focus on the slide (see Figure 80). The Camera 1 indicator displays BUSY while autofocus is running. When the Camera 1 indicator displays READY, click **AutoExpose**, then wait for the Camera 1 indicator to show READY. Click **AutoFocus** so that the Imager can focus again (this time with correct exposure settings). Note where the focus peak falls in the focus range by looking at the value that displays where the peak is located.

Figure 80 Click **AutoFocus** and **AutoExpose** so that the focus peak falls in the focus range.



3. When the Imager displays READY, click the **Acquire** button, then examine the image on the screen (see Figure 81).

Figure 81 Examine the image on the screen.

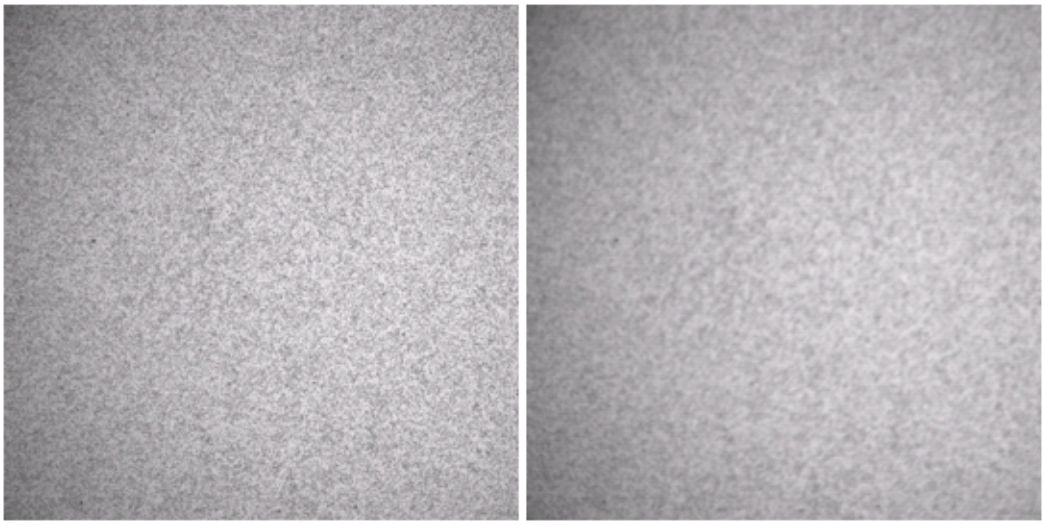


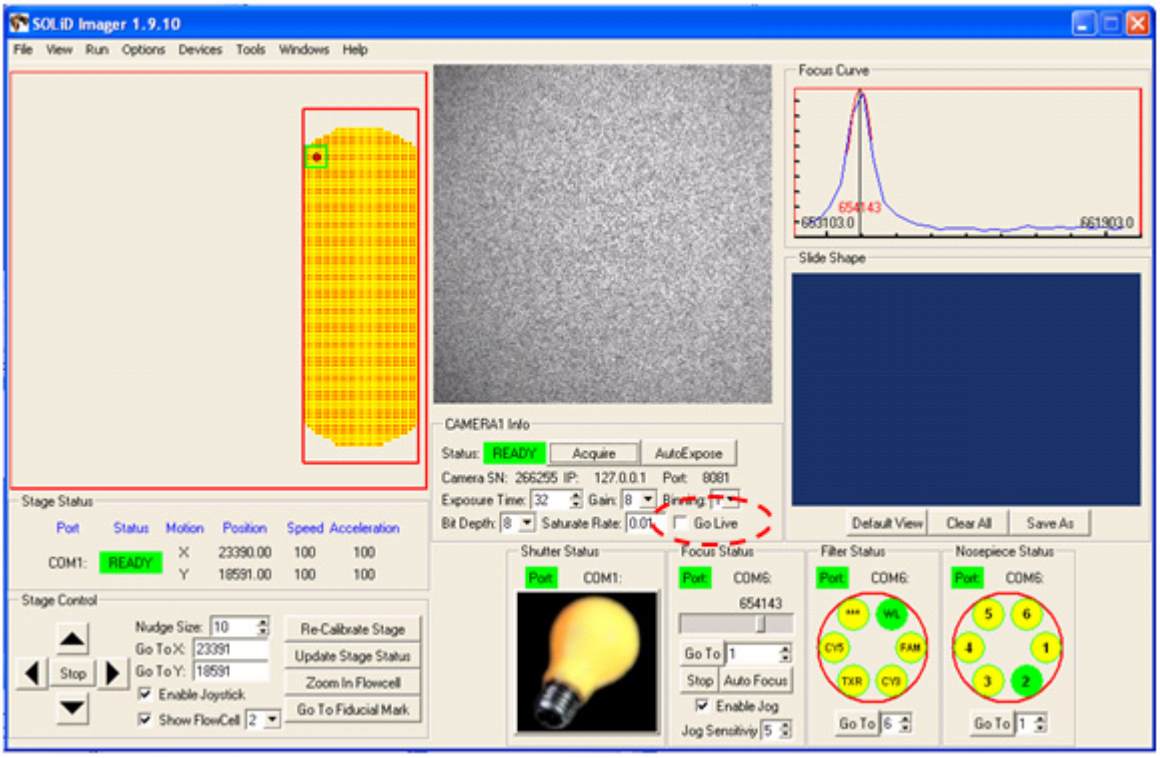
Image *in* focus | Image *out* of focus

Note: To zoom on the image, double-left-click the mouse. To un-zoom, double-right-click. To drag the image, hold down the left mouse button.

4. If the image is *in* focus, proceed to "Calculate, then set the focal range" on page 114. If the image is *out* of focus, proceed to step 5.

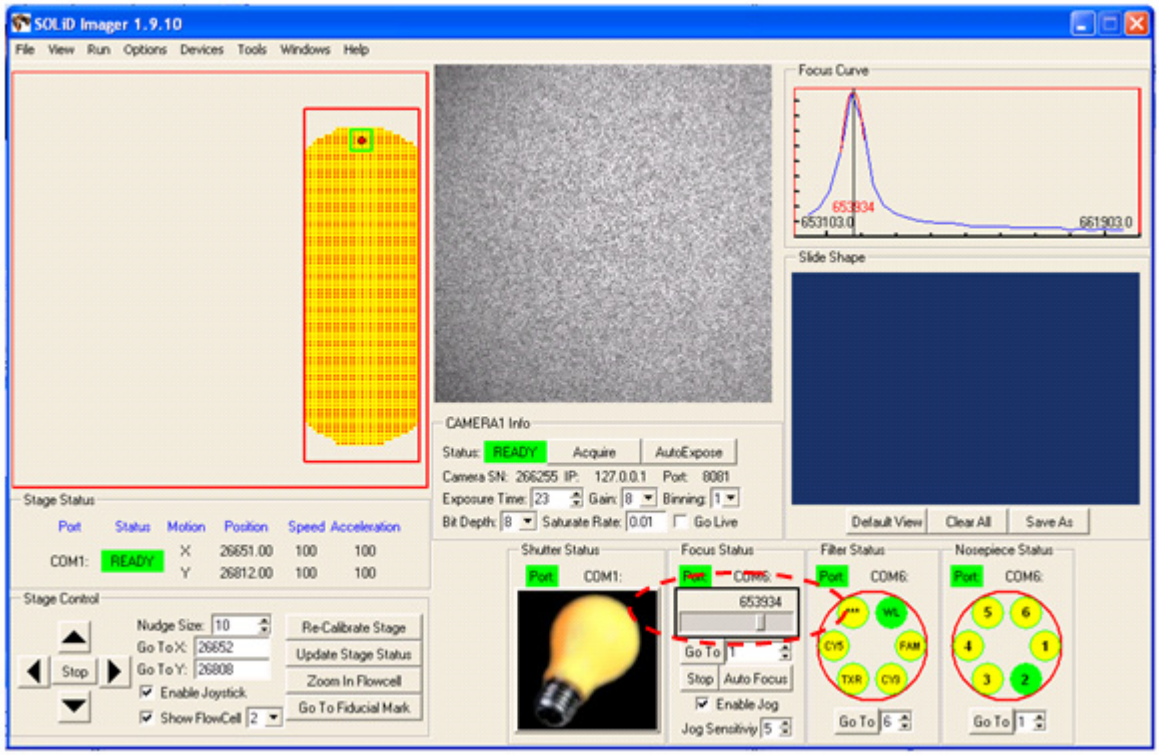
5. Check the **Go Live** check box in the Imager window. By checking the **Go Live** check box, a live image of the flowcell displays. You can use this image to manually find the focal range (see Figure 82).

Figure 82 Check **Go Live** to manually find the focal range.



6. Click the focus slider bar in the Imager window. A black box appears (see Figure 83).

Figure 83 Use the black box to change the focal distance.



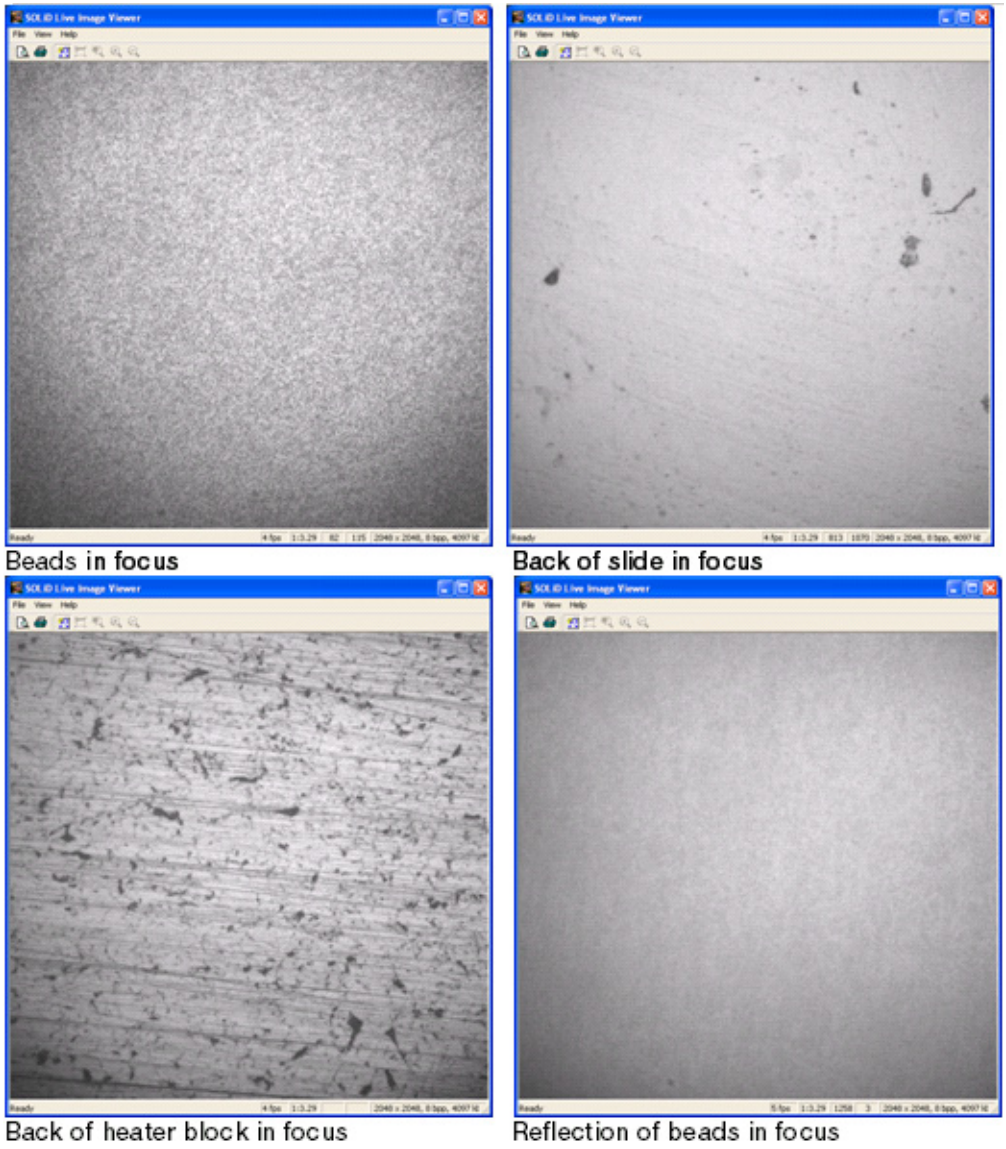
7. When the black box is visible, hold down the **Ctrl** key and hold down either the **right** or **left** arrow key on the keyboard. Holding down **Ctrl** and the arrow key (**right** or **left** arrow) simultaneously changes the focal distance in 300-count intervals.

8. While watching the live image on the screen, scan the focal distance:
   - Use the **Ctrl**+***right*** arrow keys to scan the focal distance upward. When the live image is in focus, release the keys, then record the value shown in the black box of the Imager window.
   - If an in-focus image *cannot* be found, then scan downward using the **Ctrl**+***left*** arrow keys, past the starting point, while watching the live image on the screen. When the live image is in focus, release the keys, then record the value shown in the black box of the Imager window.

   The value in the black box of the imager window when the image is in focus is the *nominal Z distance*.

   For examples of in-focus images, see Figure 84.

Figure 84 Examples of in-focus images.

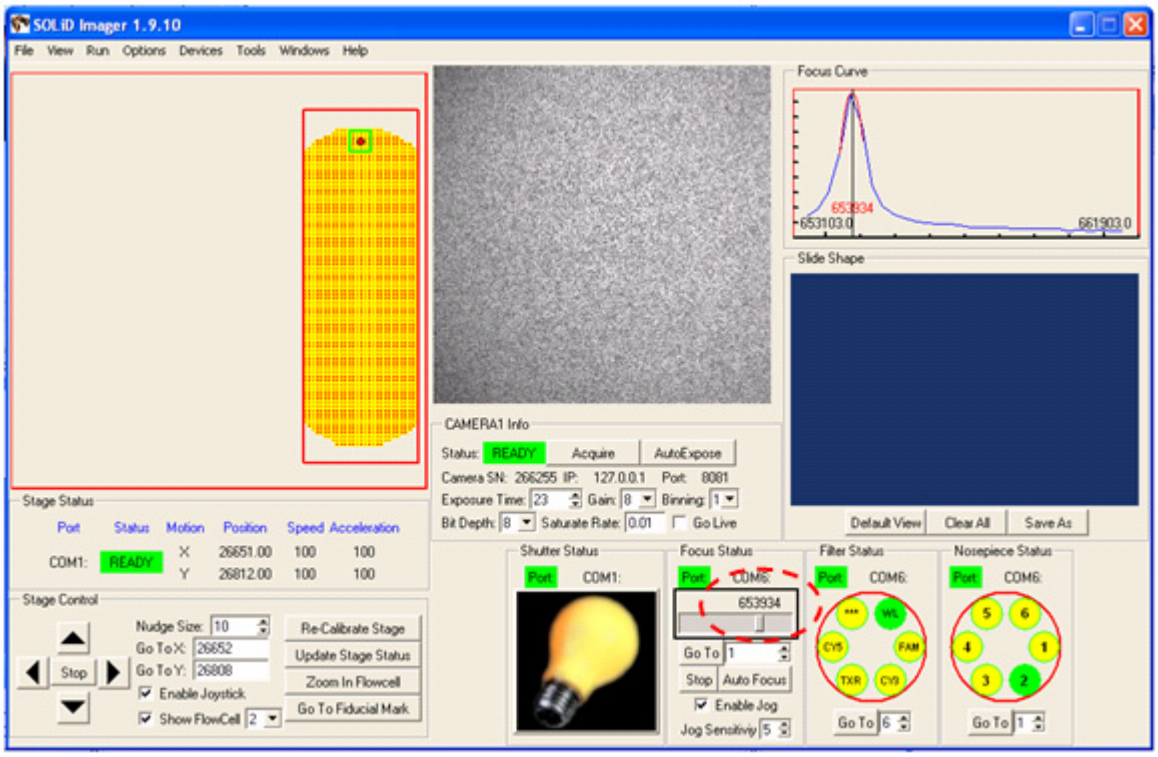


9. Uncheck the **Go Live** check box in the Imager window to close the live-image window.

Calculate, then set the focal range

1. When the image is in focus, record the value of the *Z* distance in the Focus Status pane. This is the *nominal Z distance* for this flowcell. Be sure to record the correct *Z* distance value, not the value in the GoTo window (see Figure 85).

Figure 85 Record the nominal Z distance.

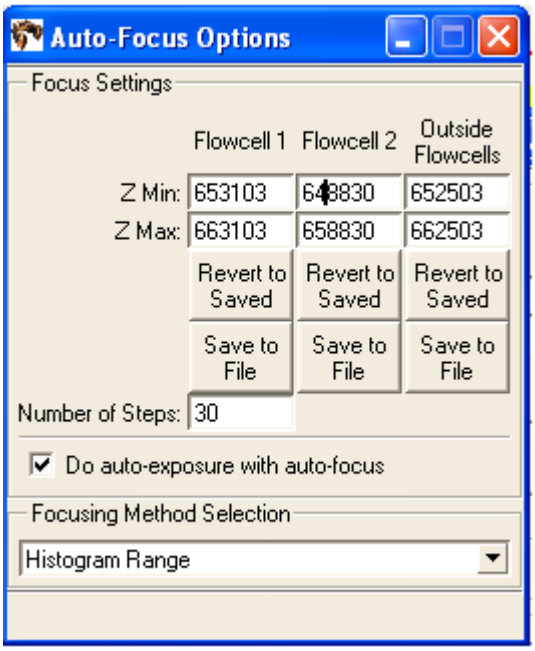


2. Choose **Options** ▸ **Focusing**.

3. Ensure that **Do auto-exposure with auto-focus** is checked. Enter a value 5000 *less than* the nominal *Z* distance for *Z* Min. Enter a value 5000 *greater than* the nominal *Z* distance for *Z* Max. Enter **30** for the Number of Steps and choose **Histogram Range** from the drop-down menu in the Focusing Method Selection pane (see Figure 86).

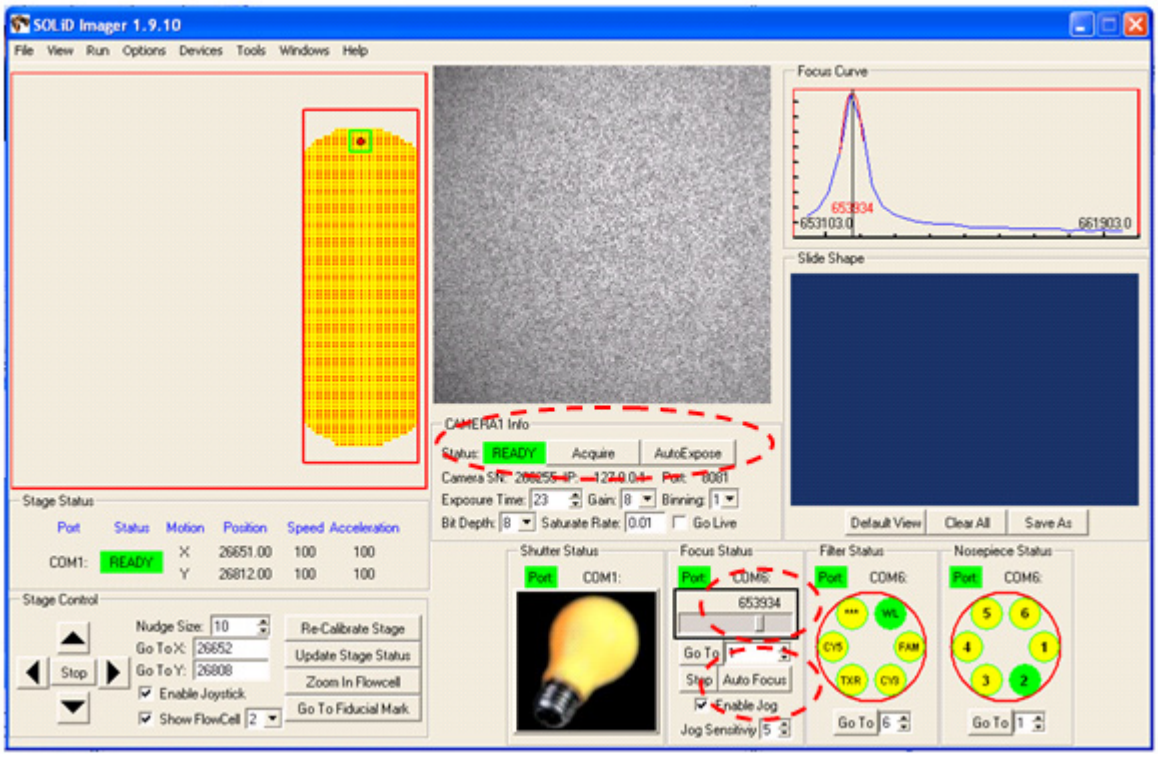
IMPORTANT! Leave the Auto-Focus Options window *open* on the Desktop. Do *not* click the **Save to File** button. Values entered in the Focusing box are used by the Imager software even if the values are not saved.

Figure 86 Enter auto-focus options.



4. Use the mouse to move the green box to the upper-left corner of the slide.
5. In the Imager window, click the **Autofocus** button, then wait about 20 seconds for the Imager to focus on the slide. The Camera 1 indicator displays BUSY while the autofocus is running. When the Camera 1 indicator displays READY, click **AutoExpose** and wait for the Camera 1 indicator to display READY. Click AutoFocus so that the Imager can focus again (this time with the correct exposure settings).
6. When the Imager displays READY, click the **Acquire** button, then confirm that the image is in focus.
7. Record the *Z* distance value from the Focus Status pane (see Figure 87).

Figure 87 Record the *Z* distance value.

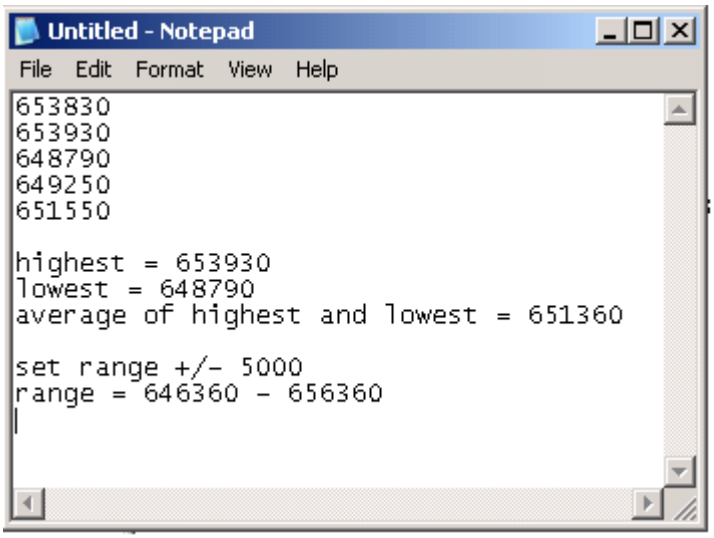


8. Open Notepad, then record the measured focal value for the spot on the slide that has been focussed.

9. Repeat steps 4–8 for the top right, bottom left, bottom right, and center of the slide. The range of these values should not exceed 5000 units. Calculate the average value of the highest and lowest spots and record this value in Notepad (see Figure 88).

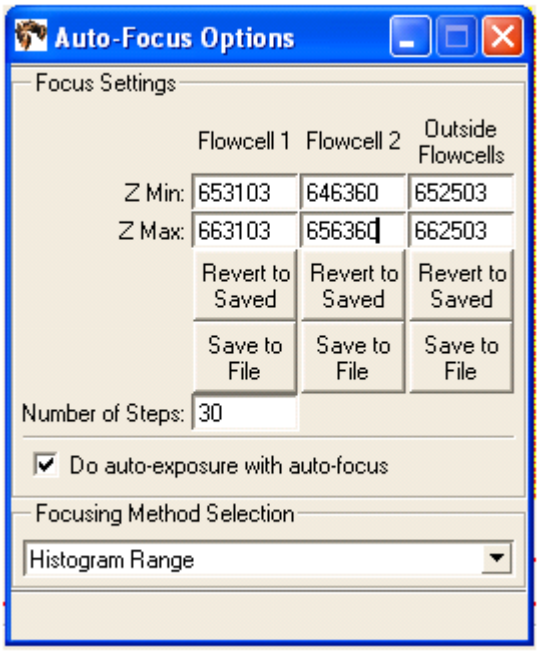
IMPORTANT! If the range of these values exceeds 5000 units, the flowcell is out of alignment. Call your Applied Biosystems Field Service Engineer to align the flowcell.

Figure 88 Record the average focal value in Notepad.



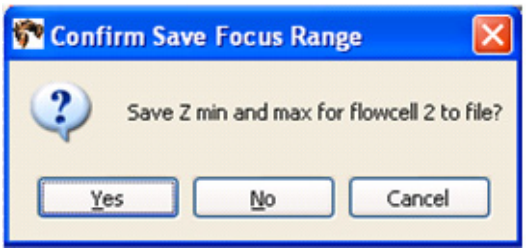
10. Subtract 5000 counts from the calculated average, then enter that value in the Z Min box. Add 5000 counts to the average, then enter that value into the Z Max box. Confirm that the Number of Steps is **30**. Click **Save to File** (see Figure 89).

Figure 89 Enter the focus settings.



11. Click **Yes** when the warning displays (see Figure 90).

Figure 90 Click **Yes** when the Save Focus Range Warning displays.



12. When the Flowcell Selection window appears, choose the number of the flowcell (**1** or **2**) that has just been measured and click **OK**. The focal range is saved.
13. (Optional) Repeat all previous steps starting from "Select the flowcell, then find the beads on the slide" on page 109.

Shut down the SOLiD® 4 Analyzer

The instrument can be shut down using the Instrument Shutdown wizard in the ICS. For instruments that have UPS, in the event of power failure, an uninterrupted power supply (UPS) is activated, UPS systematically shuts down the instrument: Analysis jobs are stopped and Linux is shut down. Slides are preserved in Storage Buffer.

Required equipment

Table 29 Required equipment: Shut down the instrument

Item	Source
UPS	Applied Biosystems 4397781 (North America) 4393695 (International)

1. Open the Instrument Shutdown wizard by choosing **Wizards** ▸ **Instrument Shutdown**.
2. Follow the instructions in the wizard.

Reset the robot position

The Robot standby script sends the robot back to home position.

1. Open the Utility Scripts menu by choosing **Tools** ▸ **Utility Scripts**.
2. Select **Robot standby**.
3. Select **Run Script**.

Store the slide in a flowcell

The Store flowcell script fills the flowcell with Storage Buffer.

1. Open the Utility Scripts menu by choosing **Tools** ▸ **Utility Scripts**.
2. Select **Store flowcell**.

3. Select **Run Script**.

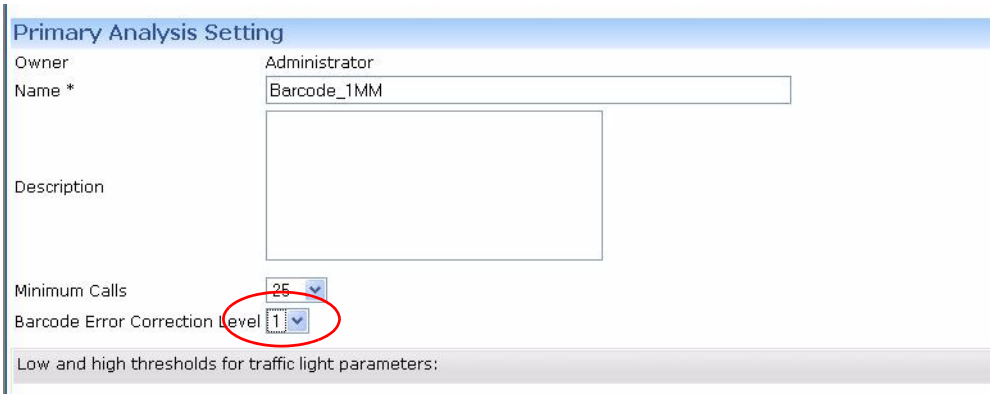
Modify the Barcode Error Correction Level

A Barcode Error Correction Level is set at 0 mismatches by default. A Barcode Error Correction Level of 1 mismatch increases the number of reads by about 10%, but lower-quality sequencing reads are included in the data.

To modify the Barcode Error Correction Level:

1. In SETS, log in as the Administrator.
2. Select **Analysis** ▸ **Primary Analysis Settings** ▸ **New**.
3. In the Barcode Error Correction Level drop-down list, select 0 or 1 (see Figure 91).
4. Save the primary analysis setting, then apply the setting to the multiplex sequencing run when the run is created.

Figure 91 In Barcode Error Correction Level, select 0 or 1.



C

On-Instrument Reagent Volumes and Reagent Strip Layouts

This appendix covers:

- Recommended fill volumes for on-instrument reagents 121
  - Paired-end sequencing (F5-BC/F5-P2 Tag) 122
  - Mate-pair sequencing (F3/R3 Tags) 123
  - Barcode sequencing (BC Tag) 123
  - Workflow analysis (WFA) 123
- Reagent strip layouts 124
  - Fragment/paired-end sequencing, F3 Tag 124
  - Mate-Pair sequencing, F3 Tag 125
  - Mate-Pair sequencing, R3 Tag. 125
  - Paired-end sequencing, F5-P2 Tag 126
  - Multiplex paired-end sequencing, F5-BC Tag 126
  - Multiplex fragment/paired-end sequencing, BC Tag 127
  - Workflow analysis (WFA) 128

Recommended fill volumes for on-instrument reagents

Volumes of on-instrument reagents

IMPORTANT! Verify that there is sufficient volume of Reset Buffer for each tag sequenced. The SOLiD® ToP Instrument Buffer contains sufficient Reset Buffer to reset two tags (see "On-Instrument Reagent Volumes and Reagent Strip Layouts" on page 121). For multiplex sequencing, a bottle of Reset Buffer is included with multiplex sequencing kits to account for sequencing the BC tag.

Table 30 Volumes of on-instrument reagents provided in the SOLiD® ToP Instrument Buffer Kit

	Volume (mL)
1X Instrument Buffer	8000†
Cleave 1 Solution	350
Cleave 2.1 Solution	350
Reset Buffer	170
Imaging Buffer	250
Ligase Buffer	120
Universal Buffer	75
Storage Buffer	825

† Volumes shown are after preparation according to the instructions in Chapter 2, "Install on-instrument reagents" on page 25.

Fragment sequencing (F3 Tag)

Table 31 Fill volumes for fragment sequencing (35 or 50 bp)

	Volume (mL)			
	35 bp		50 bp	
	1 Flowcell	2 Flowcells	1 Flowcell	2 Flowcells
Instrument Buffer	2157	4125	2949	5710
Storage Buffer	343	587	423	748
Cleave 1 Solution	124	224	169	316
Cleave 2.1 Solution	124	224	169	316
Reset Buffer	91	164	91	164
Ligase Buffer	42	74	56	101
Universal Buffer	26	41	33	55
Imaging Buffer	82	142	107	191

Paired-end sequencing (F5-BC/F5-P2 Tag)

Table 32 Fill volumes for paired-end sequencing (35 bp)

	Volume (mL)			
	F5-P2 Tag		F5-BC Tag	
	1 Flowcell	2 Flowcells	1 Flowcell	2 Flowcells
Instrument Buffer	2477	4954	2477	4954
Storage Buffer	448	896	448	896
Cleave 1 Solution	78	156	78	156
Cleave 2.1 Solution	78	156	78	156
Reset Buffer	72	145	72	145
Ligase Buffer	37	75	37	75
Universal Buffer	36	73	36	73
Imaging Buffer	68	135	68	135

Mate-pair sequencing (F3/R3 Tags)

Table 33 Fill volumes for mate-pair sequencing (25, 35, or 50 bp)

	Volume per tag (mL)					
	25 bp		35 bp		50 bp	
	1 Flowcell	2 Flowcells	1 Flowcell	2 Flowcells	1 Flowcell	2 Flowcells
Instrument Buffer	1629	3069	2157	4125	2949	5710
Storage Buffer	289	480	343	587	423	748
Cleave 1 Solution	93	163	124	224	169	316
Cleave 2.1 Solution	93	163	124	224	169	316
Reset Buffer	91	164	91	164	91	164
Ligase Buffer	33	55	42	74	56	101
Universal Buffer	21	32	26	41	33	55
Imaging Buffer	66	109	82	142	107	191

Barcode sequencing (BC Tag)

Table 34 Fill volumes for barcode sequencing (5 or 10 bp)

	Volume (mL)			
	5 bp		10 bp	
	1 Flowcell	2 Flowcells	1 Flowcell	2 Flowcells
Instrument Buffer	638	1088	883	1577
Storage Buffer	189	279	227	356
Cleave 1 Solution	32	41	47	72
Cleave 2.1 Solution	32	41	47	72
Reset Buffer	91	164	91	164
Ligase Buffer	15	19	18	25
Universal Buffer	13	14	15	19
Imaging Buffer	33	43	41	59

Workflow analysis (WFA)

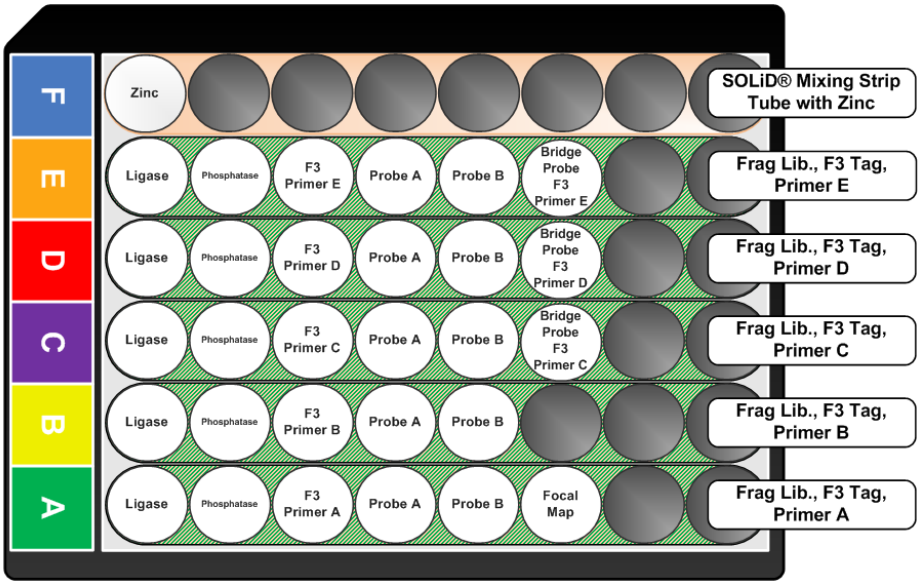
Table 35 Fill volumes for workflow analysis (WFA)

	Volume (mL)	
	1 Flowcell	2 Flowcells
Instrument Buffer	294	400
Storage Buffer	116	134
Cleave 1 Solution	0	0
Cleave 2.1 Solution	0	0

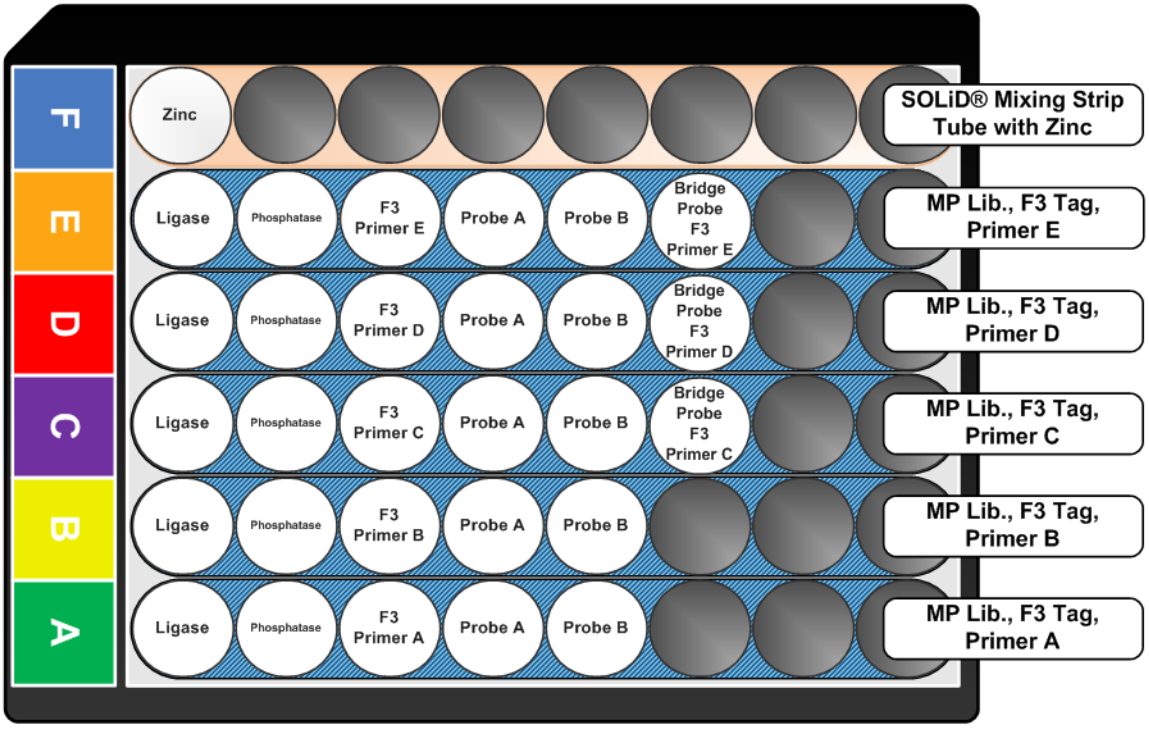
	Volume (mL)	
	1 Flowcell	**2 Flowcells**
Reset Buffer	43	67
Ligase Buffer	12	12
Universal Buffer	0	0
Imaging Buffer	26	30

Reagent strip layouts

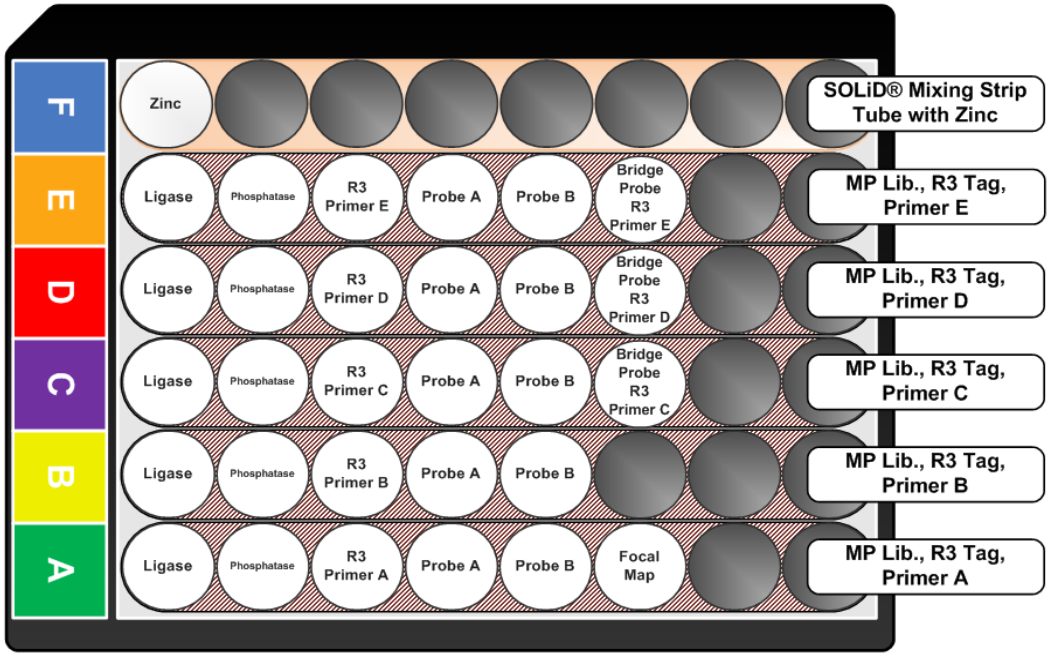
Fragment/paired-end sequencing, F3 Tag



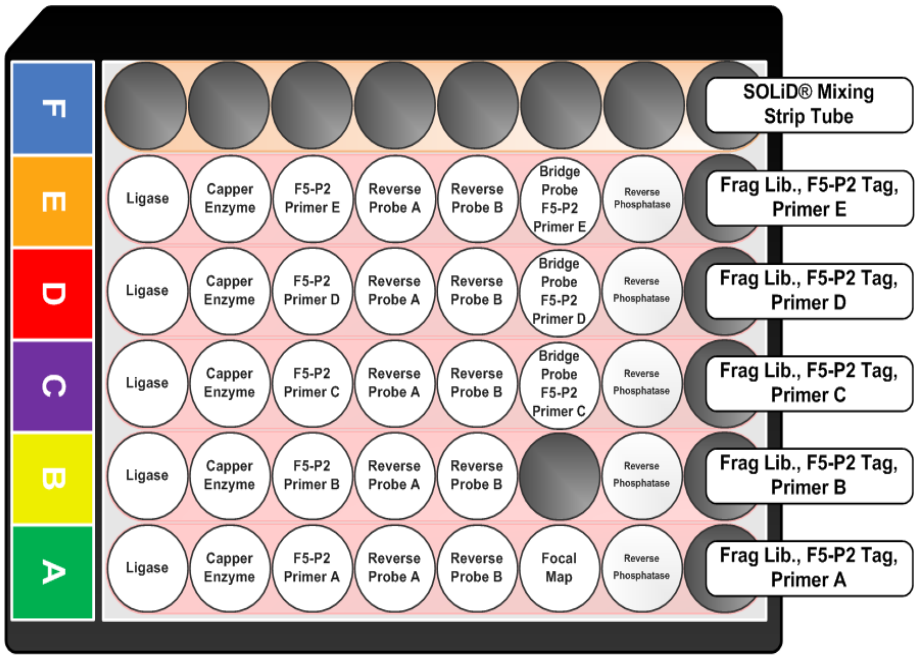
Mate-Pair sequencing, F3 Tag



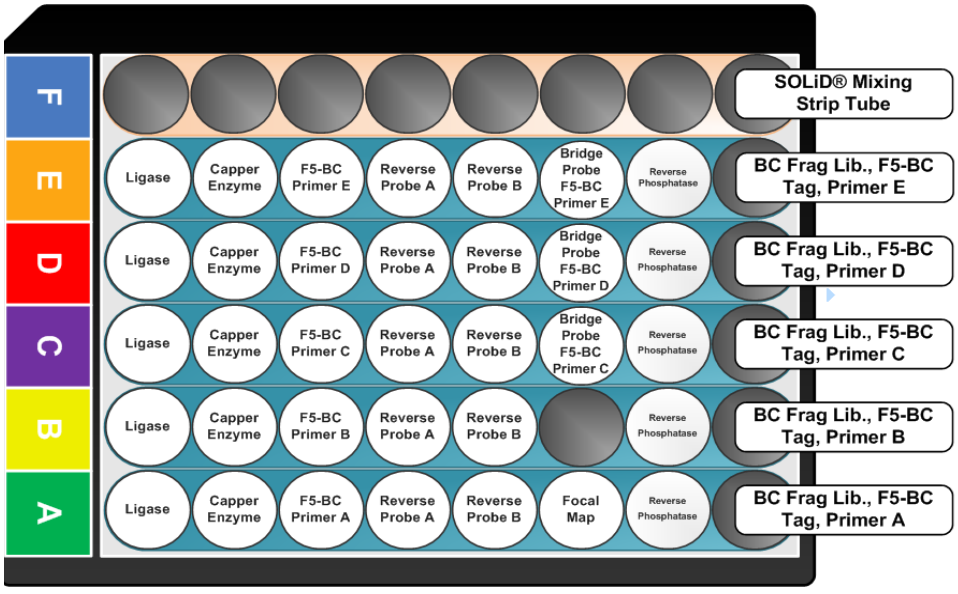
Mate-Pair sequencing, R3 Tag

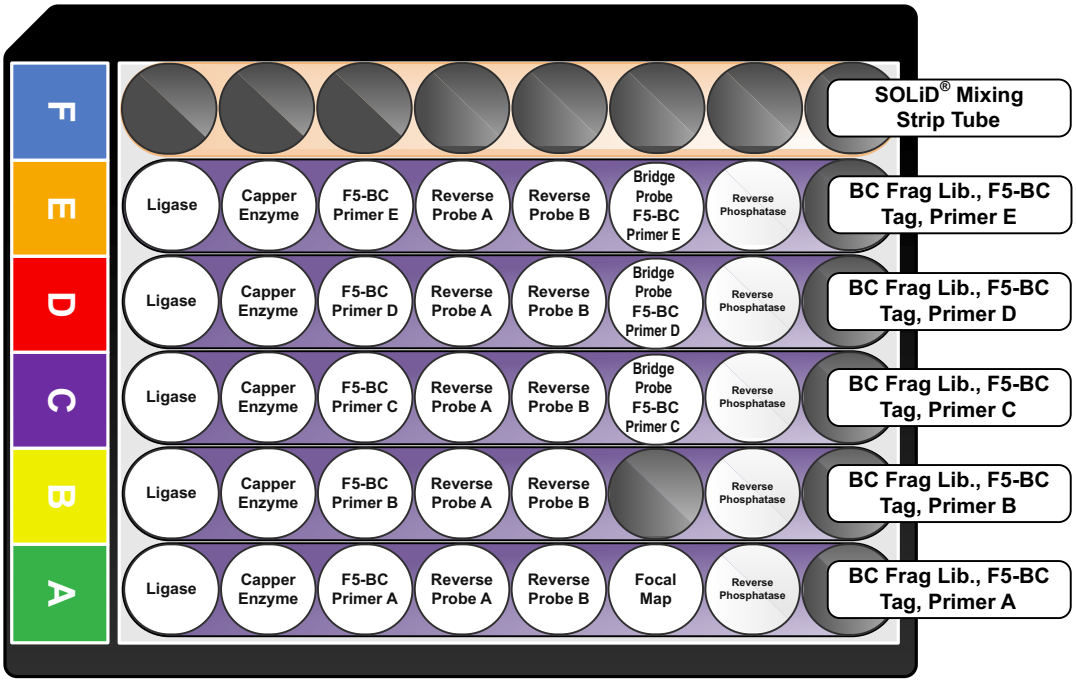


Paired-end sequencing, F5-P2 Tag

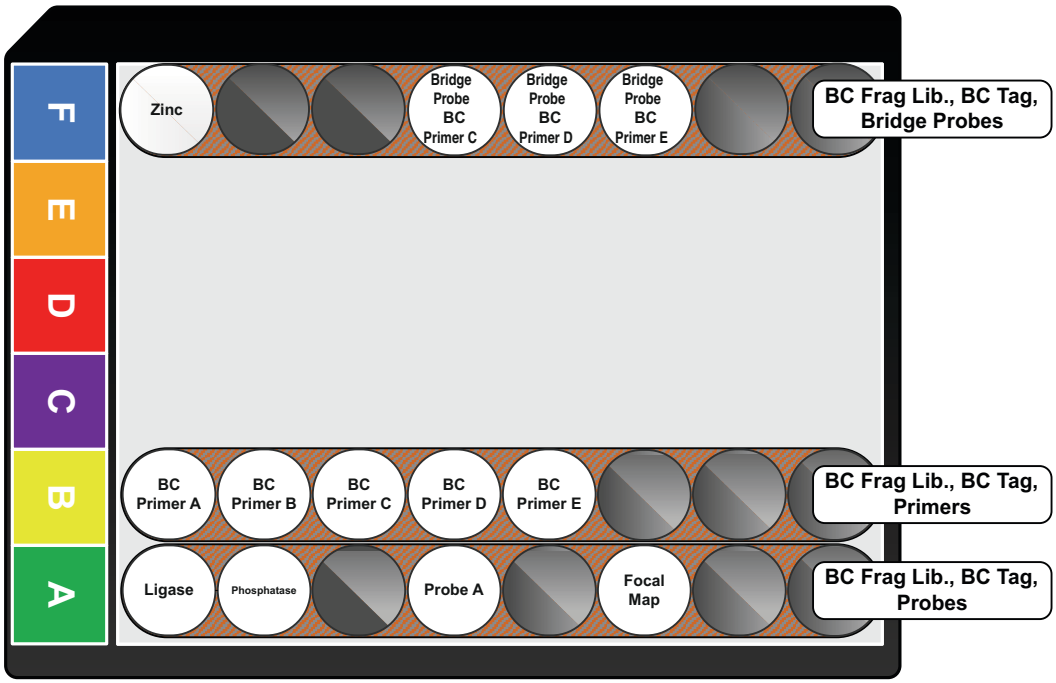


Multiplex paired-end sequencing, F5-BC Tag

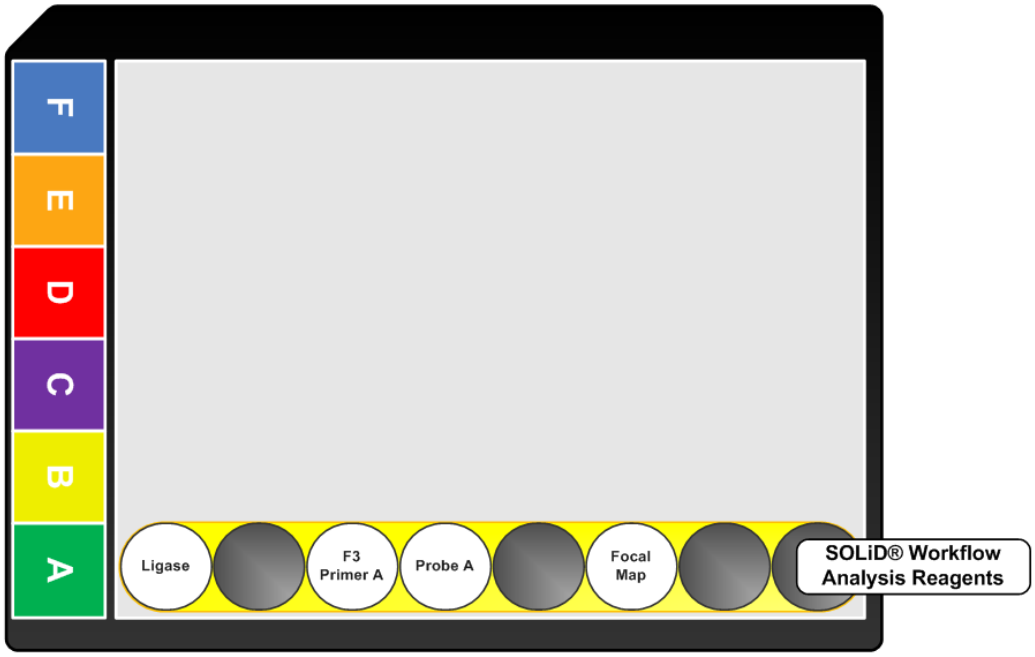




Multiplex fragment/paired-end sequencing, BC Tag



Workflow analysis (WFA)



D Instrument Process Times

This appendix covers:

- Times for individual processes 129
- Times for entire processes 130

Times for individual processes

Table 36 Times for individual processes

Process	Estimated Time (h)	Steps	Estimated time per primer (h)
Focal Map	~2–3	Reset P2 Label Image	 —
Sequencing Primers 1 & 2 and Ligation (F3/R3)	~3–4	Reset Prime Ligate Dark Ligate Phosphatase Image Cleave	~3–4 (1 cycle or 5 bp) ~15–17 (5 cycles or 25 bp) ~18–22 (7 cycles or 35 bp) ~28–33 (10 cycles or 50 bp)
Sequencing Primers 3, 4, & 5 and Ligation (F3/R3)	~4–5	Reset Prime Bridge Probe Ligate Dark Ligate Phosphatase Image Cleave	~4–5 (1 cycle or 5 bp) ~16–18 (5 cycles or 25 bp) ~19–23 (7 cycles or 35 bp) ~29–34 (10 cycles or 50 bp)
Ligation cycle without priming (F3/R3)	~2.5–3	Ligate Dark Ligate Phosphatase Image Cleave	 —

Process	Estimated Time (h)	Steps	Estimated time per primer (h)
Sequencing Primers 1 & 2 and Ligation (F5-P2/F5-BC)	~4–5	Reset Prime Ligate Dark Ligate Capper Enzyme Image Cleave RevPhosphatase	~24–30 (7 cycles or 35 bp)
Sequencing Primers 3, 4, & 5 and Ligation (F5-P2/F5-BC)	~5–6	Reset Prime Bridge Probe Ligate Dark Ligate Capper Enzyme Image Cleave RevPhosphatase	~25–30 (7 cycles or 35 bp)
Ligation cycle without priming (F5-P2/F5-BC)	~3.5–4.5	Ligate Dark Ligate Capper Enzyme Image Cleave RevPhosphatase	—

Times for entire processes

Table 37 Times for entire processes

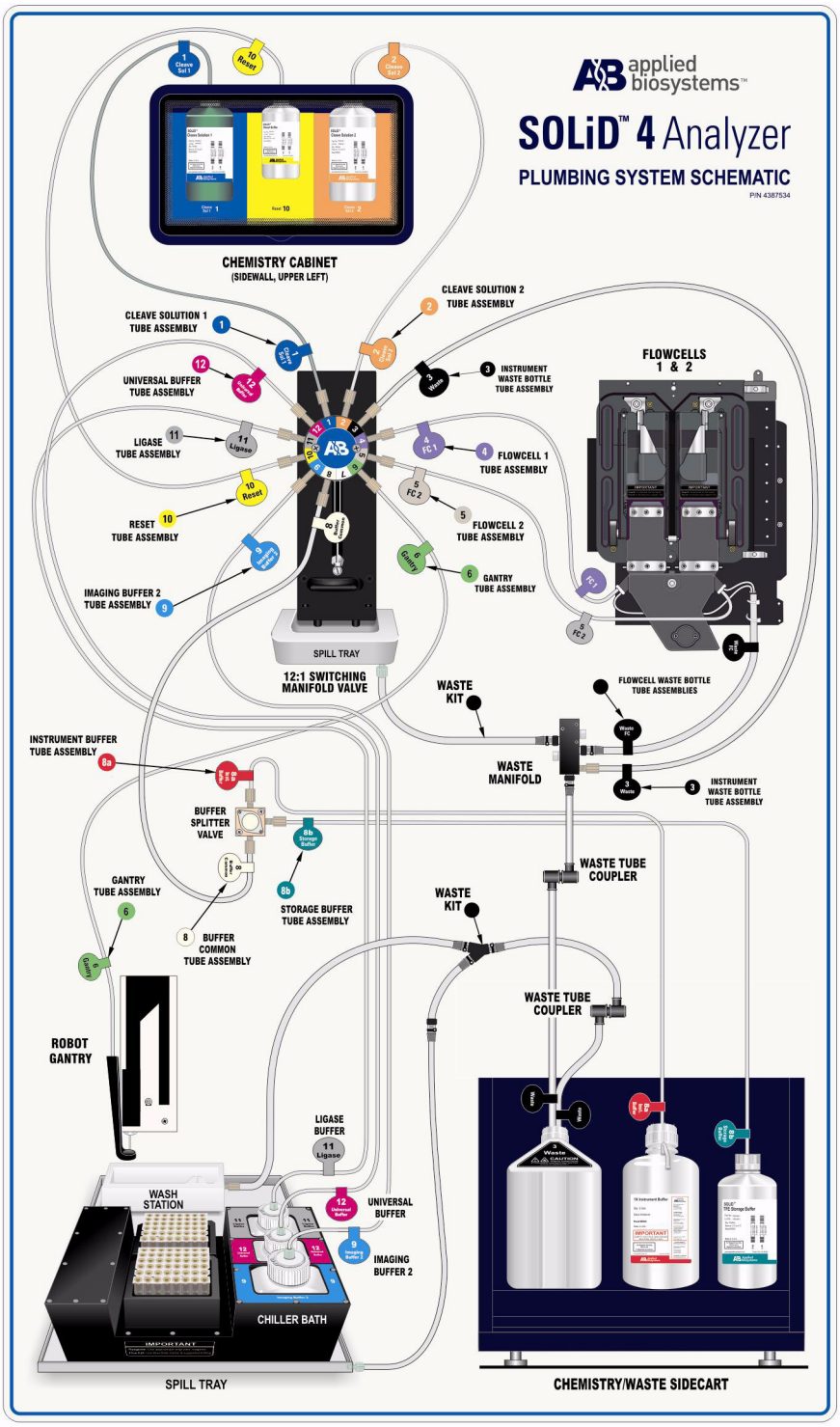
Process	Total Runtime†
Workflow Analysis (WFA)	~4–5 hours
Fragment (35 bp)	~4–4.5 days
Fragment (50 bp)	~6–7 days
Mate-paired (25/25 bp)	~7–8 days
Mate-paired (35/35 bp)	~8–9 days
Mate-paired (50/50 bp)	~13–14 days
Paired-end (50/35 bp)	~12–14 days
Multiplex fragment (35/5 bp)	~5–5.5 days
Multiplex fragment (35/10 bp)	~6–6.5 days

Process	Total Runtime†
Multiplex fragment (50/5 bp)	~7–8 days
Multiplex fragment (50/10 bp)	~8–9 days
Multiplex paired-end (50/35/5 bp)	~13–15 days
Multiplex paired-end (50/35/10 bp)	~14–16 days

† The estimated time is for a dual slide run. Estimated times may vary due to differences in imaging time.

E

SOLiD® 4 Analyzer Plumbing System Schematic



F

Checklists and Workflow Tracking Forms

This appendix covers:

- Workflow checklists: set up a workflow analysis or sequencing run 136
- Workflow tracking: set up and perform a workflow analysis (WFA) run 137
- Workflow tracking: set up a sequencing run (1-well) 138
- Workflow tracking: set up a sequencing run (4-well) 139
- Workflow tracking: set up a sequencing run (8-well) 140

Workflow checklists: set up a workflow analysis or sequencing run

	Equipment	Reagents	Preparation Steps
Deposit the beads	☐ *Covaris™ S2 System* ☐ *SOLiD® Bead Concentration Chart* ☐ *SOLiD® Deposition Chamber* ☐ *SOLiD® Slide Carrier* ☐ *NanoDrop® ND-1000* ☐ *6 Tube magnetic rack* ☐ *Vortexer* ☐ *Picofuge* ☐ *Pipettors*	☐ *1X TEX Buffer* ☐ *Deposition Buffer v2* ☐ *Double distilled filtered water* ☐ *1.5-mL LoBind Tubes* ☐ *3-mm adhesive disks* ☐ *SOLiD™ System Sequencing Slide* ☐ *Slide Prep Reagent* ☐ *Filtered pipettor tips*	—
Install on-instrument reagents	☐ *SOLiD® 4 Analyzer* ☐ *8-L reagent bottle* ☐ *Graduated cylinder* ☐ *Magnetic stir bar* ☐ *Pipettors*	☐ *10X Instrument Buffer* ☐ *Storage Buffer* ☐ *1X T4 DNA Ligase Buffer (Parts 1 and 2)* ☐ *Universal Buffer (Parts 1 and 2)* ☐ *Imaging Buffer (Parts 1 and 2)* ☐ *Cleave Solution 1* ☐ *Cleave Solution 2.1 (Parts 1 and 2)* ☐ *Reset Buffer* ☐ *Glycerol* ☐ *Double-distilled water*	—
Install slide(s)	☐ *SOLiD® 4 Analyzer* ☐ *Pipettors* ☐ *Allen wrench* ☐ *SOLiD® Slide Storage Chamber (optional)*	☐ *Deposition Buffer v2* ☐ *Filtered pipettor tips* ☐ *70% ethanol* ☐ *Kimwipes®* *Slide Storage Buffer*	—
Install reagent strip(s)	☐ *Tabletop centrifuge*	☐ *96-well square-well storage plate*	☐ *Thaw appropriate reagent strip(s)*

Workflow tracking: set up and perform a workflow analysis (WFA) run

Slide:				**Date:**
Run:				**Date:**
Sample Name				
Sample information (pre-WFA)				
A600				
Concentration (beads/µL)				
Deposition Volume (µL)				
WFA report				
P2_rfu				
P2_Exp				
P2_Gain				
P1#				
P2#				
P2#/P1# ratio				
N2S				
On Axis				
Titration Metric				
Sample information (post-WFA)				
P2 Concentration (beads/µL)				
Volume Left				
Beads Left				

Lot numbers	
Slide	
Deposition Buffer v2	
Slide Prep Reagent	
Instrument Buffer	
Storage Buffer	
T4 Ligase Buffer Part 1	
T4 Ligase Buffer Part 2	
Universal Buffer Part 1	
Universal Buffer Part 2	
Imaging Buffer Part 1	
Imaging Buffer Part 2	
Cleave Solution 1	
Cleave Solution 2.1 Part 1	
Cleave Solution 2.1 Part 2	
Reset Buffer	
Slide Storage Buffer	
Workflow Analysis Reagents	

Workflow tracking: set up a sequencing run (1-well)

Slide:		**Date:**
Run:		**Date:**
Sample Name		
Sample information		
A600		
Concentration (beads/µL)		
Deposition Volume (µL)		
Volume Left		
Beads Left		
Lot numbers		
Slide		
Deposition Buffer v2		
Slide Prep Reagent		
Instrument Buffer		
Storage Buffer		
T4 Ligase Buffer Part 1		
T4 Ligase Buffer Part 2		
Universal Buffer Part 1		
Universal Buffer Part 2		
Imaging Buffer Part 1		
Imaging Buffer Part 2		
Cleave Solution 1		
Cleave Solution 2.1 Part 1		
Cleave Solution 2.1 Part 2		
Reset Buffer		
Slide Storage Buffer		
F3 Tag Sequencing Kit		
F3 – Primer A		
F3 – Primer B		
F3 – Primer C		
F3 – Primer D		
F3 – Primer E		
F3 – Mixing Strip Tube		
R3/F5-P2/F5-BC Tag Sequencing Kit		
R3/F5-P2/F5-BC – Primer A		
R3/F5-P2/F5-BC – Primer B		
R3/F5-P2/F5-BC – Primer C		
R3/F5-P2/F5-BC – Primer D		
R3/F5-P2/F5-BC – Primer E		
R3/F5-P2/F5-BC – Mixing Strip Tube		
BC Tag Sequencing Kit		
BC – Bridge Probes		
BC – Primers		
BC – Probes		

Workflow tracking: set up a sequencing run (4-well)

Slide:				**Date:**
Run:				**Date:**
Samples 1 to 4				
Sample Name				
Sample information				
A600				
Concentration (beads/µL)				
Deposition Volume (µL)				
Volume Left				
Beads Left				

Lot numbers	
Slide	
Deposition Buffer v2	
Slide Prep Reagent	
Instrument Buffer	
Storage Buffer	
T4 Ligase Buffer Part 1	
T4 Ligase Buffer Part 2	
Universal Buffer Part 1	
Universal Buffer Part 2	
Imaging Buffer Part 1	
Imaging Buffer Part 2	
Cleave Solution 1	
Cleave Solution 2.1 Part 1	
Cleave Solution 2.1 Part 2	
Reset Buffer	
Slide Storage Buffer	
F3 Tag Sequencing Kit	
F3 – Primer A	
F3 – Primer B	
F3 – Primer C	
F3 – Primer D	
F3 – Primer E	
F3 – Mixing Strip Tube	
R3/F5-P2/F5-BC Tag Sequencing Kit	
R3/F5-P2/F5-BC – Primer A	
R3/F5-P2/F5-BC – Primer B	
R3/F5-P2/F5-BC – Primer C	
R3/F5-P2/F5-BC – Primer D	
R3/F5-P2/F5-BC – Primer E	
R3/F5-P2/F5-BC – Mixing Strip Tube	
BC Tag Sequencing Kit	
BC – Bridge Probes	
BC – Primers	
BC – Probes	

Workflow tracking: set up a sequencing run (8-well)

Slide:				**Date:**
Run:				**Date:**
Samples 1 to 4				
Sample Name				
Sample Information				
A600				
Concentration (beads/µL)				
Deposition Volume (µL)				
Volume Left				
Beads Left				
Samples 5 to 8				
Sample Name				
Sample information				
A600				
Concentration (beads/µL)				
Deposition Volume (µL)				
Volume Left				
Beads Left				

Lot numbers	
Slide	
Deposition Buffer v2	
Slide Prep Reagent	
Instrument Buffer	
Storage Buffer	
T4 Ligase Buffer	
Universal Buffer Part 1	
Universal Buffer Part 2	
Imaging Buffer Part 1	
Imaging Buffer Part 2	
Cleave Solution 1	
Cleave Solution 2.1 Part 1	
Cleave Solution 2.1 Part 2	
Reset Buffer	
Slide Storage Buffer	
F3 Tag Sequencing Kit	
F3 – Primer A	
F3 – Primer B	
F3 – Primer C	
F3 – Primer D	
F3 – Primer E	
F3 – Mixing Strip Tube	
R3/F5-P2/F5-BC Tag Sequencing Kit	
R3/F5-P2/F5-BC – Primer A	
R3/F5-P2/F5-BC – Primer B	
R3/F5-P2/F5-BC – Primer C	
R3/F5-P2/F5-BC – Primer D	
R3/F5-P2/F5-BC – Primer E	
R3/F5-P2/F5-BC – Mixing Strip Tube	
BC Tag Sequencing Kit	
BC – Bridge Probes	
BC – Primers	
BC – Probes	

G

The Covaris® S2 System

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  - Set the chiller 141
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- Covaris® S2 Programs 142
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Operation notes

Fill the tank

Fill the tank with fresh deionized water to the proper fill line. The water should cover the visible part of the tube.

Degas the water

Degas the water for 30 minutes. To maintain degassed water, keep the pump continuously on during operation and sample processing.

Set the chiller

Set the chiller temperature to between 2–5°C to ensure that the temperature reading in the water bath displays 5°C. The circulated water chiller should be supplemented with 20% ethylene glycol.

Perform required maintenance of the Covaris® S2 System

The Covaris® S2 System requires regular maintenance to work properly. Perform the tasks in the table below (see Table 38):

Table 38 Required maintenance of the Covaris® S2 System

Required maintenance task	Frequency to perform task
Degas water for 30 minutes prior to use	Before every use
Change water	Daily
Clean with bleach	Every two weeks

Covaris® S2 Programs

Covalent Declump 1

Table 39 Covalent Declump 1: 1 cycle Treatment 1 followed by 1 cycle Treatment 2

	Treatment 1	Treatment 2
Duty Cycle	2%	5%
Intensity	6	9
Cycles/Burst	100	100
Time	5 sec	30 sec
Target wattage power performance estimate (W)†	4	15

† Not programmed.

Covalent Declump 3

Table 40 Covalent Declump 3: 3 cycles Treatment 1 followed by 1 cycle Treatment 2

	Treatment 1	Treatment 2
Duty Cycle	2%	5%
Intensity	6	9
Cycles/Burst	100	100
Time	5 sec	30 sec
Target wattage power performance estimate (W)†	4	15

† Not programmed.

H Instrument Warranty Information

This appendix covers:

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Computer configuration

Applied Biosystems supplies or recommends certain configurations of computer hardware, software, and peripherals for use with its instrumentation. Applied Biosystems reserves the right to decline support for or impose extra charges for supporting nonstandard computer configurations or components that have not been supplied or recommended by Applied Biosystems. Applied Biosystems also reserves the right to require that computer hardware and software be restored to the standard configuration prior to providing service or technical support. For systems that have built-in computers or processing units, installing unauthorized hardware or software may void the Warranty or Service Plan.

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Applied Biosystems warrants that all standard components of the SOLiD® 4 Analyzer, IKA® ULTRA-TURRAX® Tube Drive, the Covaris® S2 System, APC UPS, and the recirculating chiller will be free of defects in materials and workmanship for a period of one (1) year from the date the warranty period begins. Applied Biosystems will repair or replace, at its discretion, all defective components during this warranty period. Applied Biosystems warrants the Genomic Solutions HydroShear® DNA Shearing Device will be free of defects in materials and workmanship for a period of one (1) year from the date the warranty period begins. Applied Biosystems will replace a defective HydroShear® DNA Shearing Device during the warranty period. The following parts of the HydroShear® DNA Shearing Device are use- replaceable and not covered by the warranty on the HydroShear® DNA Shearing Device: shearing assembly, syringes, syringe adapters, syringe shields, and output tubing. Applied Biosystems reserves the right to use new, repaired, or refurbished instruments or components for warranty and post-warranty service agreement replacements. Repair or replacement of products or components that are under warranty does not extend the original warranty period.

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With the exception of consumable and maintenance items, replaceable products or components used on or in the instrument are themselves warranted to be free of defects in materials and workmanship for a period of ninety (90) days.

Applied Biosystems warrants that chemicals and other consumable products will be free of defects in materials and workmanship when received by the buyer, but not thereafter, unless otherwise specified in documentation accompanying the product.

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Applied Biosystems at its sole discretion may refuse to provide buyer with support or service for buyer's use of Covaris® S2 in a method not described in a SOLiD® System protocol.

Applied Biosystems does not warrant that the operation of the instrument or its operating software will be uninterrupted or be error-free.

Warranty period effective date

Any applicable warranty period under these sections begins on the earlier of the date of installation or ninety (90) days from the date of shipment for hardware and software installed by Applied Biosystems personnel. For all hardware and software installed by the buyer or anyone other than Applied Biosystems, and for all other products, the applicable warranty period begins the date the product is delivered to the buyer.

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Warranty exceptions

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Parts in contact with any liquid are considered wetted and may be deemed user-replaceable and not be covered by the above warranties, including, but not limited to, seals, filters, gaskets, shearing assemblies, valves, syringes, syringe adapters, syringe shields, and output tubing.

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Damages, claims, and returns

Damages

If shipping damage to the product is discovered, contact the shipping carrier and request inspection by a local agent. Secure a written report of the findings to support any claim. Do not return damaged goods to Applied Biosystems without first securing an inspection report and contacting Applied Biosystems Technical Support for a Return Authorization (RA) number.

Claims

After a damage inspection report is received by Applied Biosystems, Applied Biosystems will process the claim unless other instructions are provided.

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Do not return any material without prior notification and authorization.

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I Life Technologies End User Software License Agreement

This appendix covers:

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J

Safety

WARNING! GENERAL SAFETY. Using this product in a manner not specified in the user documentation may result in personal injury or damage to the instrument or device. Ensure that anyone using this product has received instructions in general safety practices for laboratories and the safety information provided in this document.

- Before using an instrument or device, read and understand the safety information provided in the user documentation provided by the manufacturer of the instrument or device.
- Before handling chemicals, read and understand all applicable Safety Data Sheets (SDSs) and use appropriate personal protective equipment (gloves, gowns, eye protection, etc). To obtain SDSs, see the "Documentation and Support" section in this document.

Symbols on this instrument

Symbols may be found on the instrument to warn against potential hazards or convey important safety information. In this document, the symbol is used along with user attention words described in the "About This Guide" section to highlight important safety information. The following table lists the meaning of these symbols.

Symbol	English
	Caution, risk of danger Consult the manual for further safety information.
	Caution, hazardous waste. Refer to SDS(s) and local regulations for handling and disposal.
	Caution, hot surface
	Caution, risk of electrical shock
	Moving parts
	Potential slipping hazard
	Potential overhead hazard

Symbol	English
	On
	Off
	On/Off
	Standby
	Earth (ground) terminal
	Protective conductor terminal (main ground)
	Terminal that can receive or supply alternating current or voltage
	Terminal that can receive or supply alternating or direct current or voltage
	Do not dispose of this product in unsorted municipal waste **CAUTION!** To minimize negative environmental impact from disposal of electronic waste, do not dispose of electronic waste in unsorted municipal waste. Follow local municipal waste ordinances for proper disposal provision and contact customer service for information about responsible disposal options.

Safety alerts on this instrument

Additional text may be used with one of the symbols described above when more specific information is needed to avoid exposure to a hazard. See the following table for safety alerts found on the instrument.

Safety label	Location on Instrument
CAUTION! Hot Surfaces	Flow cell cover and inside arc lamp box
CAUTION! Replace only with CERMAX tx300f, 300 w lamp.	Arc lamp box cover
WARNING! HOT! Do not remove lamp until 15 minutes after disconnecting power supply.	Arc lamp box cover
CAUTION! Crush/pinch hazard	Syringe pump

Safety information for instruments not manufactured by Life Technologies

Some of the accessories provided as part of the instrument system are not designed or built by Life Technologies. Consult the manufacturer's documentation for the information needed for the safe use of these products.

Instrument safety

General

CAUTION! Do not remove instrument protective covers. If you remove the protective instrument panels or disable interlock devices, you may be exposed to serious hazards including, but not limited to, severe electrical shock, laser exposure, crushing, or chemical exposure.

CAUTION! Solvents and Pressurized fluids. Wear eye protection when working with any pressurized fluids. Use caution when working with any polymeric tubing that is under pressure:

- Extinguish any nearby flames if you use flammable solvents.
- Do not use polymeric tubing that has been severely stressed or kinked.
- Do not use polymeric tubing with tetrahydrofuran or nitric and sulfuric acids.
- Be aware that methylene chloride and dimethyl sulfoxide cause polymeric tubing to swell and greatly reduce the rupture pressure of the tubing.
- Be aware that high solvent flow rates (~40mL/min) may cause a static charge to build up on the surface of the tubing and electrical sparks may result.

Physical injury

CAUTION! Moving and Lifting Injury. The instrument is to be moved and positioned only by the personnel or vendor specified in the applicable site preparation guide.

Improper lifting can cause painful and permanent back injury.

Things to consider before lifting or moving the instrument or accessories:

- Depending on the weight, moving or lifting may require two or more persons.
- If you decide to lift or move the instrument after it has been installed, do not attempt to do so without the assistance of others, the use of appropriate moving equipment, and proper lifting techniques.
- Ensure you have a secure, comfortable grip on the instrument or accessory.
- Make sure that the path from where the object is to where it is being moved is clear of obstructions.
- Do not lift an object and twist your torso at the same time. Keep your spine in a good neutral position while lifting with your legs.
- Participants should coordinate lift and move intentions with each other before lifting and carrying.
- For smaller packages, rather than lifting the object from the packing box, carefully tilt the box on its side and hold it stationary while someone else slides the contents out of the box.

CAUTION! Moving Parts. Moving parts can crush, pinch and cut. Keep hands clear of moving parts while operating the instrument. Disconnect power before servicing.

Electrical

WARNING! Fuse Installation. Before installing the instrument, verify that the fuses are properly installed and the fuse voltage matches the supply voltage. Replace fuses only with the type and rating specified for the unit. Improper fuses can damage the instrument wiring system and cause a fire.

WARNING! Ensure appropriate electrical supply. For safe operation of the instrument:

- Plug the system into a properly grounded receptacle with adequate current capacity.
- Ensure the electrical supply is of suitable voltage.
- Never operate the instrument with the ground disconnected. Grounding continuity is required for safe operation of the instrument.

WARNING! Power Supply Line Cords. Use properly configured and approved line cords for the power supply in your facility.

Cleaning and decontamination

CAUTION! CLEANING AND DECONTAMINATION.
Before using a cleaning or decontamination method other than those recommended by the manufacturer, verify with the manufacturer that the proposed method will not damage the equipment.

Safety and electromagnetic compatibility (EMC) standards

The instrument design and manufacture complies with the standards and requirements for safety and electromagnetic compatibility as noted in the following table:

Safety

Reference	Description
EU Directive 2006/95/EC	European Union "Low Voltage Directive"
IEC 61010-1 EN 61010-1 UL 61010-1 CSA C22.2 No. 61010-1	*Safety requirements for electrical equipment for measurement, control, and laboratory use – Part 1: General requirements*
IEC 61010-2-010 EN 61010-2-010	*Safety requirements for electrical equipment for measurement, control and laboratory use – Part 2-010: Particular requirements for laboratory equipment for the heating of materials*

Reference	Description
IEC 61010-2-081 EN 61010-2-081	*Safety requirements for electrical equipment for measurement, control and laboratory use – Part 2-081: Particular requirements for automatic and semi-automatic laboratory equipment for analysis and other purposes*
IEC 60825-1 EN 60825-1	*Safety of laser products – Part 1: Equipment classification and requirements*
21 CFR 1040.10 and 1040.11 as applicable	U.S. FDA Health and Human Services (HHS) "Radiological health performance standards for laser products" and "Radiological health performance standards for specific purpose laser products"

EMC

Reference	Description
Directive 2004/108/EC	European Union "EMC Directive"
EN 61326-1	*Electrical Equipment for Measurement, Control and Laboratory Use – EMC Requirements – Part 1: General Requirements*
FCC Part 18 (47 CFR)	U.S. Standard "Industrial, Scientific, and Medical Equipment"
AS/NZS 2064	*Limits and Methods of Measurement of Electromagnetic Disturbance Characteristics of Industrial, Scientific, and Medical (ISM) Radiofrequency Equipment*
ICES-001, Issue 3	*Industrial, Scientific and Medical (ISM) Radio Frequency Generators*

Environmental design

Reference	Description
Directive 2002/96/EC	European Union "WEEE Directive" – Waste electrical and electronic equipment
Directive 2002/95/EC	European Union "RoHS Directive" – Restriction of hazardous substances in electrical and electronic equipment
Directive 2006/66/EC	European Union "Battery Directive"
MII Order #39	PRC "Management Methods for Controlling Pollution by Electronic Information Products"

Chemical safety

WARNING! GENERAL CHEMICAL HANDLING. To minimize hazards, ensure laboratory personnel read and practice the general safety guidelines for chemical usage, storage, and waste provided below, and consult the relevant SDS for specific precautions and instructions:

- Read and understand the Safety Data Sheets (SDSs) provided by the chemical manufacturer before you store, handle, or work with any chemicals or hazardous materials. To obtain SDSs, see the "Documentation and Support" section in this document.
- Minimize contact with chemicals. Wear appropriate personal protective equipment when handling chemicals (for example, safety glasses, gloves, or protective clothing).
- Minimize the inhalation of chemicals. Do not leave chemical containers open. Use only with adequate ventilation (for example, fume hood).
- Check regularly for chemical leaks or spills. If a leak or spill occurs, follow the manufacturer's cleanup procedures as recommended in the SDS.
- Handle chemical wastes in a fume hood.
- Ensure use of primary and secondary waste containers. (A primary waste container holds the immediate waste. A secondary container contains spills or leaks from the primary container. Both containers must be compatible with the waste material and meet federal, state, and local requirements for container storage.)
- After emptying a waste container, seal it with the cap provided.
- Characterize (by analysis if necessary) the waste generated by the particular applications, reagents, and substrates used in your laboratory.
- Ensure that the waste is stored, transferred, transported, and disposed of according to all local, state/provincial, and/or national regulations.
- **IMPORTANT!** Radioactive or biohazardous materials may require special handling, and disposal limitations may apply.

WARNING! HAZARDOUS WASTE (from instruments). Waste produced by the instrument is potentially hazardous. Follow the guidelines noted in the preceding General Chemical Handling warning.

WARNING! 4L Reagent and Waste Bottle Safety. Four-liter reagent and waste bottles can crack and leak. Each 4-liter bottle should be secured in a low-density polyethylene safety container with the cover fastened and the handles locked in the upright position.

Biological hazard safety

WARNING! Potential Biohazard. Depending on the samples used on this instrument, the surface may be considered a biohazard. Use appropriate decontamination methods when working with biohazards.

WARNING! BIOHAZARD. Biological samples such as tissues, body fluids, infectious agents, and blood of humans and other animals have the potential to transmit infectious diseases. Follow all applicable local, state/provincial, and/or national regulations. Wear appropriate protective equipment, which includes but is not limited to: protective eyewear, face shield, clothing/lab coat, and gloves. All work should be conducted in properly equipped facilities using the appropriate safety equipment (for example, physical containment devices). Individuals should be trained according to applicable regulatory and company/institution requirements before working with potentially infectious materials. Read and follow the applicable guidelines and/or regulatory requirements in the following:

In the U.S.:

- U.S. Department of Health and Human Services guidelines published in Biosafety in Microbiological and Biomedical Laboratories found at: **www.cdc.gov/biosafety**
- Occupational Safety and Health Standards, Bloodborne Pathogens (29 CFR§1910.1030), found at: **www.access.gpo.gov/nara/cfr/waisidx_01/29cfr1910a_01.html**
- Your company's/institution's Biosafety Program protocols for working with/handling potentially infectious materials.
- Additional information about biohazard guidelines is available at: **www.cdc.gov**

In the EU:

Check local guidelines and legislation on biohazard and biosafety precaution and refer to the best practices published in the World Health Organization (WHO) Laboratory Biosafety Manual, third edition, found at: **www.who.int/csr/resources/publications/biosafety/WHO_CDS_CSR_LYO_2004_11/en/**

Documentation and Support

Related documentation

The following related documents are shipped with the system:

Document	Part number	Description
Applied Biosystems SOLiD® 4 System Library Preparation Guide	4445673	Provides procedures for preparing libraries.
Applied Biosystems SOLiD® 4 System Library Preparation Quick Reference Card	4445674	Provides brief, step-by-step procedures for preparing libraries.
Applied Biosystems SOLiD® 4 System Templated Bead Preparation Guide	4448378	Describes how to prepare templated beads by emulsion PCR (ePCR), required before sequencing on the SOLiD® 4 System.
Applied Biosystems SOLiD® 4 System Templated Bead Preparation Quick Reference Card	4448329	Provides brief, step-by-step procedures for preparing templated beads by emulsion PCR (ePCR), required before sequencing on the SOLiD® 4 System.
Applied Biosystems SOLiD® 4 System Instrument Operation Quick Reference Card	4448380	Provides brief, step-by-step procedures for loading and running the SOLiD® 4 System.
Applied Biosystems SOLiD® 4 System Site Preparation Guide	4448639	Provides all the information that you need to set up the SOLiD® 4 System.
Applied Biosystems SOLiD® 4 System SETS Software User Guide	4448411	Provides an alternate platform to monitor runs, modify settings, and reanalyze previous runs that are performed on the SOLiD® 4 System.
Applied Biosystems SOLiD® 4 System ICS Software Help	—	Describes the software and provides procedures for common tasks (see the Instrument Control Software).
BioScope™ Software for Scientists Guide	4448431	Provides a bioinformatics analysis framework for flexible application analysis (data-generated mapping, SNPs, count reads) from sequencing runs.

Document	Part number	Description
Working with SOLiDBioScope.com™ Quick Reference Card	4452359	Provides an online suite of software tools for Next Generation Sequencing (NGS) analysis. SOLiDBioScope.com™ leverages the scalable resources of cloud computing to perform computation-intensive NGS data processing.
Applied Biosystems SOLiD® 4 System Software Integrated Workflow Quick Reference Guide	4448432	Describes the relationship between the software comprising the SOLiD 4 platform and provides quick step procedures on operating each software to perform data analysis.
Applied Biosystems SOLiD®4 System Product Selection Guide	4452360	Provides a quick guide to the sequencing kits you need to perform fragment, paired-end, mate-pair, multiplex fragment, and multiplex paired-end sequencing.
Applied Biosystems SOLiD® System SOLiD® 3 Plus to SOLiD® 4 System User Documentation Changes	4451929	Provides a brief summary of changes made between the SOLiD® 3 Plus System documentation and the SOLiD® 4 System documentation.
Applied Biosystems SOLiD® 4 Upgrade Checklist	4449773	Provides a checklist to ensure that all necessary preparations are made before upgrading to the SOLiD® 4 System and provides a list of consumables you can order.

Note: For additional documentation, see "Limited product warranty" on page 163.

Obtaining SDSs

Safety Data Sheets (SDSs) are available from **www.lifetechnologies.com/support**.

Note: For the SDSs of chemicals not distributed by Life Technologies, contact the chemical manufacturer.

Obtaining support

For the latest services and support information for all locations, go to:

www.lifetechnologies.com/support

At the website, you can:

- Access worldwide telephone and fax numbers to contact Technical Support and Sales facilities
- Search through frequently asked questions (FAQs)
- Submit a question directly to Technical Support
- Search for user documents, SDSs, vector maps and sequences, application notes, formulations, handbooks, certificates of analysis, citations, and other product support documents
- Obtain information about customer training
- Download software updates and patches

Limited product warranty

Life Technologies Corporation and/or its affiliate(s) warrant their products as set forth in the Life Technologies' General Terms and Conditions of Sale found on Life Technologies' website at www.lifetechnologies.com/termsandconditions. If you have any questions, please contact Life Technologies at www.lifetechnologies.com/support.

Glossary

barcode	Unique sequence identifier added to the sample during library construction
barcoded fragment library	Fragment library with a barcode sequence appended to the 3′ end of the sheared DNA fragments
BC tag	Barcode tag
bead count	The number of beads detected in a panel
bead signal	The average signal intensity of every pixel associated with a bead
best beads	Number of beads that meet a stringent set of criteria based on spectral purity and intensity
F3 tag	Tag to be sequenced using primers specific to the P1 Adaptor sequence
F5-P2 tag	Tag to be sequenced using reverse ligation chemistry and using primers specific to the P2 Adaptor sequence (for paired-end sequencing of non-barcoded fragment libraries)
F5-BC tag	Tag to be sequenced using reverse ligation chemistry and using primers specific to the Internal Adaptor sequence (for paired-end sequencing of barcoded fragment libraries)
fragment library	Library consisting of a sheared DNA fragment with P1 and P2 Adaptors ligated to the 5′ end and 3′ end respectively
good beads	The number of beads that meet criteria (less stringent than best beads criteria) based on spectral purity and intensity
image signal	The average signal intensity of every pixel whether or not it is associated with a bead or not
internal adaptor	Double-stranded oligonucleotide located between two tags to be sequenced
library	Set of DNA tags prepared from the same biological sample to be sequenced on the SOLiD® System
mappable beads	Beads with template that map back to the reference genome
mate-paired library	Library consisting of two DNA tags a known distance apart linked by an internal adaptor with P1 and P2 Adaptors ligated to the 5′ end and 3′ end, respectively
multiplexing	Method to analyze multiple biological samples in a single spot using barcodes

N2S plot	Plot indicating noise-to-signal for each dye
on-axis beads	The frequency of template-positive beads that meet a defined threshold of spectral purity and signal intensity after a single ligation step
optimal titration point	Library template concentration that gives the best sequencing results
P1 Adaptor	Double-stranded oligonucleotide ligated at the 5′ end of the library
P2 Adaptor	Double-stranded oligonucleotide ligated at the 3′ end of the library
P2#/P1# ratio	The frequency of template-positive beads (P2#) relative to total beads (P1#) deposited on the slide; this metric is also referred to as "% P2 Positive" value
P2-positive beads	SOLiD® P1 DNA beads with fully extended and amplified template
pulse-spin	Place the tube in a picofuge and spin for a few seconds to bring down any beads or liquid stuck on the walls of the tube
R3 tag	Tag to be sequenced using primers specific to the Internal Adaptor sequence
remove the supernatant	Use a pipette to carefully remove the liquid from the tube without disturbing any beads
resuspend the beads	The beads can be resuspended in one of two ways: • Gently pipet the solution up and down until the beads are suspended. Using a slower speed to aspirate and expel the solution minimizes the amount of beads that stick to the inside of the pipette tip. • Vortex the solution until all of the beads are suspended. Place the beads in a picofuge and pulse-spin for a few seconds to bring down any beads stuck on the walls of the tube. Do not over-spin the beads or the beads aggregate into a pellet.
Satay plot	Indicator of spectral purity and signal intensity of the beads
sonicate the beads	Place the tube containing the beads in the appropriate tube holder, then place in the Covaris® S2 System; afterwards, run the appropriate program
tag	A length of DNA to be sequenced
templated bead preparation	Process of adding library template to beads by emulsion PCR, enriching the beads to remove beads without template, then modifying the 3′ end of the template on the beads to prepare for bead deposition and sequencing
templated beads	SOLiD® P1 DNA Beads with amplified library template attached
titration	Library template concentration used to prepare an emulsion

titration metric	Product of %P2 positive beads and the On-Axis beads; the titration that generates the highest titration metric value is the optimal titration point for a given library
usable beads	Number of beads that are called during color-calling
workflow analysis (WFA) run	Type of run on the SOLiD® system in which a small portion of templated beads are deposited and analyzed to test for templated bead quality

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